Stress response of boreal woodland caribou, moose, and wolves to disturbance in eastern Manitoba

by

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Abstract

Disturbance can provoke a chronic (long-term) stress response in wildlife, and can contribute to population declines. I examined the stress response of three wildlife species that may be responding to anthropogenic disturbance in eastern Manitoba. Boreal woodland caribou (*Rangifer tarandus caribou*; hereinafter referred as caribou) are listed as threatened under the Manitoba Endangered Species and Ecosystems Act, moose (*Alces alces*) populations have declined in southeastern Manitoba, and although wolf (*Canis lupus*) populations are stable in Manitoba, individual variation in physiological stress could still reflect differential exposure to disturbance. To examine the chronic stress response of caribou, moose, and wolves to disturbance in eastern Manitoba, I measured cortisol concentrations in hair. Caribou cortisol concentrations were greatest for the three most southern populations (Owl-Flintstone, Atiko, and Bloodvein) and increased with decreasing home range size. The best explanation for individual variation in caribou cortisol concentrations was the proportion of their home range that had been logged in the previous 6-21 years. For moose, however, disturbance did not affect cortisol concentrations, but cortisol concentrations were higher in moose killed by wolves than moose collected by humans, suggesting that chronic stress in moose is linked to poor body condition and increased vulnerability to wolf predation. Wolf cortisol concentrations increased in 2012 and 2013 compared to 2011 following increased harvest pressure, and were higher in females. However, neither winter severity nor variation in wolf diet, estimated using stable isotope analysis, affected wolf cortisol concentrations. Determining chronic stress in caribou, moose, and wolves can be important for monitoring population health, particularly with changes in disturbance regimes and implementation of management strategies that may broadly affect wildlife and their habitats.

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This thesis is dedicated to my dear friend, Amy Gilbert. Thanks for sharing your passion for the environment, wisdom, and wonderful friendship. I know you would be very proud of me, just as I was very proud of you. You are constantly missed and never forgotten. Love you so much.

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Thesis format

This thesis is in manuscript format. I wrote Chapters 1, 2, and 3 as individual manuscripts each with their own abstract, introduction, methods, results, discussion, conclusions, references, tables, and figures. I also wrote an overall introduction and conclusion for my thesis, which summarizes background information, conclusions, and management implications. At the end of my thesis are two appendices; Appendix A provides lab method validation and Appendix B examines the link between moose stress response and body condition.

Boreal woodland caribou, moose, and wolf hair samples, along with location data and other sample information, were provided by Manitoba Conservation and Water Stewardship. Wolf and prey stable isotope ratios were provided by Danielle Fox. I performed all hormone, stable isotope, spatial, and data analyses, and wrote all chapters with guidance from my committee.

Thesis introduction

Disturbance is a distinct, temporary event that alters population, community, or ecosystem structure, and/or changes the physical landscape and resource availability (Pickett et al. 1989; Cyr et al. 2009; Dornelas 2010). Common natural and anthropogenic (human impact) disturbances affecting wildlife include fire, flooding, infrastructure development, and logging (Dornelas 2010). Forests have always been shaped by natural disturbances, such as fire, but anthropogenic disturbances are surpassing natural disturbances, and are contributing to species loss and extinction worldwide (Drever et al. 2006; Cyr et al. 2009; Zwolak 2009). In particular, disturbances causing habitat loss and fragmentation can force wildlife to travel across less favourable habitat or concentrate populations into smaller areas, increasing their risk to predation, human hunting, and disease (Andrén 1994; Jalkotzy et al. 1997; St-Laurent et al. 2009). Disturbances such as vehicles, noises, and lights can also directly affect wildlife (Anderson 1978; Forman and Alexander 1998; Trombulak and Frissell 2000; Kociolek et al. 2011), and anthropogenic corridors, such as roads, pipelines, and power lines, can grant humans and predators with greater access to wildlife (Brody and Pelton 1989; Jalkotzy et al. 1997; Forman and Alexander 1998; James and Stuart-Smith 2000).

Alternatively, disturbances can attract wildlife by providing habitat or movement corridors. In particular, moose (*Alces alces*) are often attracted to browse on early successional vegetation found on the edge of corridors (Peek 1974; Gasaway et al. 1989) and coyotes (*Canis latrans*) are attracted to forage on small mammals found along highways (Jalkotzy et al. 1997; Tigas et al. 2002). Wildlife also use corridors to disperse, migrate, and search for mates (Beier 1995; Jalkotzy et al. 1997; Forman and Alexander 1998). Indeed, wolves (*Canis lupus*) and coyotes use packed snowmobile routes to reduce travel costs in winter (Kolbe et al. 2007; Gese

et al. 2013), and wolves can also use corridors to access boreal woodland caribou (*Rangifer tarandus caribou*) in treed muskeg and fen-bog habitat (James and Stuart-Smith 2000).

Disturbance and other influences on boreal woodland caribou

Declines in boreal woodland caribou populations over several decades have prompted their listing as threatened under the federal Species at Risk Act (SARA). In Manitoba, boreal woodland caribou (hereinafter referred as caribou) are also listed as threatened under the Manitoba Endangered Species and Ecosystems Act. Threats to caribou are considered interconnected, but vary based on impact (Manitoba Conservation 2006; Manitoba Boreal Woodland Caribou Management Committee 2015). For instance, habitat loss alone affects caribou populations, but the combination of habitat loss and increased predator access to caribou through anthropogenic corridors has an even greater impact on their populations. Current threats to caribou populations include habitat loss and fragmentation, predation, hunting, and transmission of disease and parasites (James and Stuart-Smith 2000; Dyer et al. 2001; Weclaw and Hudson 2004; Wasser et al. 2011; Manitoba Boreal Woodland Caribou Management Committee 2015). Threats in the future might also include greater fire frequency as a consequence of climate change (Manitoba Boreal Woodland Caribou Management Committee 2015).

Wolf predation is considered a primary contributor to caribou decline (Seip 1992; McLoughlin et al. 2003; James et al. 2004; Manitoba Boreal Woodland Caribou Management Committee 2015). Caribou and wolves have coexisted for thousands of years, but caribou populations have only declined recently, suggesting that industrial development has affected the caribou's ability to separate itself from wolves and other ungulate prey (McLoughlin et al. 2003; James et al. 2004; Latham et al. 2011a and b). Wolves are typically associated with more evenly

dispersed and abundant ungulate prey, including white-tailed deer (*Odocoileus virginianus*) and moose (Bergerud and Elliott 1998; Latham et al. 2011b; Wasser et al. 2011), which inhabit areas disturbed by anthropogenic development or natural disturbance (Monthey 1984; Gasaway et al. 1989). However, habitat loss and fragmentation can increase wolf access to caribou by altering their movements so that they expand their range into caribou habitat (Wittmer et al. 2005).

Caribou will attempt to minimize spatial overlap with other ungulates and wolves by occupying mature, large stands of undisturbed forest or fen-bog complexes (Bradshaw et al. 1995; Stuart-Smith et al. 1997). They will also separate themselves from alternative prey by living in small groups and by distancing themselves from other caribou during calving season (Bergerud et al. 1984; Bergerud and Page 1987; Stuart-Smith et al. 1997); however, when caribou avoid disturbed areas, they might crowd and increase their vulnerability to predators, hunting, and disease (Seip 1991; Dyer et al. 2001). Indeed, interactions between caribou and white-tailed deer can result in transmission of the meningeal worm (*Parelaphostrongylus tenuis*). This nematode parasite spreads from white-tailed deer to caribou when caribou forage on vegetation that contains infected gastropods, the intermediate host of *P. tenuis* (Anderson 1972). Although *P. tenuis* is benign in deer, it causes disease in caribou, making them easy targets for predators (Anderson 1972; Trainer 1973).

Disturbance and other influences on moose

Moose populations have repeatedly declined across North America, particularly in the southern extent of their range (Lankester 2010). In southern Manitoba, moose populations declined by 65% between 2000 and 2010 (Leavesley 2010). A number of factors could be contributing to moose decline, including parasitism, hunting, wolf predation, human disturbance, reduced habitat quality, and climate change (Gasaway et al. 1992; Kunkel and Pletscher 2000;

Murray et al. 2006; Shura and Roth 2013). Parasitism is thought to be an important contributor to moose decline in southeastern Manitoba (Shura and Roth 2013; Daniel Dupont, Manitoba Conservation and Water Stewardship, personal communication). Greater parasite transmission can result from habitat degradation that alters host-parasite dynamics and allows for the invasion of pathogens into new environments (Daszak et al. 2000; Murray et al. 2006). In particular, disturbance can increase overlap between moose and deer populations, putting moose at greater risk of contracting parasites from deer (Anderson 1972). Transmission of the meningeal worm from deer to moose is thought to be a primary contributor to recent (1995 - 2008) moose declines in southeastern Manitoba (Lankester 2010). This nematode affects the central nervous system of the moose, eventually causing death (Anderson 1972). The cestode *Echinococcus granulosus* also uses moose as an intermediate host and is present in southeastern Manitoba (Friesen and Roth 2016). Infection of *E. granulosus* in moose affects their lung capacity and ability to maintain long periods of exertion, particularly when pursued by wolves (Joly and Messier 2004). Winter tick (*Dermacentor albipictus*) can also contribute to large die offs of moose (Samuel 2007). The tick feeds on the blood of moose, and since moose are not proficient at grooming ticks from their skin, increased tick numbers can cause moose to groom their coat excessively, thinning their fur and making them susceptible to severe, cold temperatures (McLaughlin and Addison 1986).

Disturbance, including anthropogenic corridors, logged, and burned areas, can affect moose either negatively or positively (Kunkel and Pletscher 2000). Disturbed areas with minimal human presence might be beneficial for moose since open habitat permits the growth of shrubby vegetation that moose prefer (Monthey 1984; Gasaway et al. 1989). However, moose might be less inclined to occupy disturbed habitat where hunting, traffic, and noise levels are high

(Forman and Alexander 1998; Laurian et al. 2008). Indeed, roads with high human traffic could agitate moose and contribute to moose-vehicle collisions, especially when moose search for salt pools or forage along highways (Dussault et al. 2007; Laurian et al. 2008; Bartzke et al. 2015).

Wolves could also be contributing to moose declines in southeastern Manitoba, particularly since moose are a prominent prey source in wolf summer diet (Mocker 2015). Habitat loss and fragmentation, including linear corridors, can improve wolf hunting efficiency (James and Stuart-Smith 2000; Kunkel and Pletscher 2000). Few studies have investigated how wolves affect moose through the use of linear corridors, but Kunkel and Pletscher (2000) found that moose kills were most prominent in areas where elevation and snow depth were low, thereby facilitating wolf movements. They also found that wolves used roads to improve their hunting efficiency; however, the risk of mortality from human disturbance, such as snowmobiling and hunting, prevailed over the benefits of using roads to find prey. Alternatively, they found that moose were at greater risk of wolf predation around streams and trails since these corridors improved wolf hunting efficiency away from human disturbance.

Hunting is also considered an important factor contributing to moose population decline in eastern Manitoba. Crichton et al. (2004) found that a moose population increased from approximately 37 to 142 after road access was denied and hunting was prohibited for seven years in the Happy Lake area of eastern Manitoba. Similarly, Rempel et al. (1997) and Potvin et al. (2005) found that moose densities increased in logged areas when hunting was restricted.

Disturbance and other influences on wolves

Wolf populations are stable throughout most of Canada (52,000 – 60,000; Hayes and Gunson 1995), including Manitoba (Daniel Dupont, Manitoba Conservation and Water Stewardship, unpublished data), but increasing human disturbance, such as traffic, development,

infrastructure, and harvest, can affect wolves (Kuzyk et al. 2004; Houle et al. 2010). Anthropogenic corridors can improve wolf hunting efficiency and reduce their travel costs in winter; however, high levels of human traffic and activity can deter wolves (Mladenoff et al. 1995; Kunkel and Pletscher 2000; Kuzyk et al. 2004; Whittington et al. 2005). Wolf habitat also declines as human populations, farming practices, and development increase (Mech 1996; Boitani 2003).

Increased harvest pressure can disturb the strong social dynamics of wolves (Borg et al. 2015; Bryan et al. 2015; Molnar et al. 2015). Wolf harvest is a management technique that has been commonly employed to mitigate predation pressure on domestic animals (Boitani 2003) and declining prey, including moose and caribou (Gasaway et al. 1983; Mech 1995; Boertje et al. 1996). However, wolves are very social animals, and removing individuals from a pack, particularly an alpha member, risks disturbing the strong social unity of the pack and its ability to hunt, reproduce, defend kill sites, and transfer knowledge among generations (Brainerd et al. 2008; Borg et al. 2015; Bryan et al. 2015; Molnar et al. 2015).

Prey abundance, availability, and defense strategies can also affect wolves and cause mortality. Wolves are opportunistic generalists and will eat a range of foods, including ungulates, small mammals, fish, and garbage (Peterson and Ciucci 2003). Wolves may exhibit prey preference, depending on abundance of certain prey and their body size, anti-predator defenses, and vulnerability (Fuller and Keith 1980; Potvin et al. 1988; Mech and Peterson 2003; Garrott et al. 2007). Wolves might target ungulates, such as deer and moose, in winter when deep snow hinders their movements (i.e., wolves with lighter foot loading; Kelsall 1969; Nelson and Mech 1986; Fuller 1991). When a preferred prey source declines, becomes unavailable, or difficult to attain, wolves could suffer malnutrition (Mech 1977), but will often adjust by switching their diet

(Garrott et al. 2007). Indeed, wolves might consume smaller prey or scavenge more during mild winters when ungulate vulnerability decreases (Fuller 1991; Mech and Peterson 2003). Wolves might also switch their diet based on pack size; large wolf packs (i.e., four wolves) could take down larger prey, whereas small packs (e.g., two wolves) or solitary wolves often hunt small prey or scavenge (Hayes et al. 2000). Large wolf packs become particularly important when attacking large, aggressive prey, such as moose, which can inflict injury and cause mortality (Hayes et al. 2000; Mech and Peterson 2003).

Intra-specific strife and aggression among wolves can also cause mortality, particularly when wolves defend or fight for territory (Mech and Boitani 2003). Competition for food resources and breeding rights can also occur within a pack (Mech and Boitani 2003; Sands and Creel 2004). Juveniles typically forage on carcasses after the breeding pair finishes, but conflicts can arise when food resources become low (Packard 2003). Furthermore, breeding competition can occur when offspring remain within a pack following sexual maturity (Derix et al. 1993; Packard 2003).

Wolves are also susceptible to disease and parasitism in eastern Manitoba (Friesen and Roth 2016). Wolves can endure a range of parasites (e.g., protozoans, trematodes, cestodes, and nematodes) without harm, but when malnutrition, disease, or a virus weakens a wolf, parasites can be fatal (Mech 1977). Wolves are susceptible to the mange mite (*Sarcoptes scabiei*), which in advanced stages can cause emaciation and death (Kreeger 2003). Diseases, such as rabies, canine distemper, and canine parvovirus, can also kill wolves (Kreeger 2003).

Stress response of boreal woodland caribou, moose, and wolves

The mechanisms underlying the effects of disturbance on wildlife are not well known, but studies have recognized that disturbance can cause a stress response in wildlife that may

contribute to population declines (Boonstra and Singleton 1993; Boonstra et al. 1998; Wikelski and Cooke 2006; Charbonnel et al. 2008). When a mammal experiences a stressful situation, the hypothalamic-pituitary-adrenal axis releases glucocorticosteroids (primarily cortisol in ungulates and canids) from the adrenal axis into the blood (Boonstra and Singleton 1993; Boonstra 2004; Reeder and Kramer 2005). The release of steroid hormones within an organism can temporarily cause increased foraging, suppression of the reproductive axis, and gluconeogenesis, which is the production of glucose by breakdown of glycogen, fats, and proteins (Boonstra and Singleton 1993; Boonstra 2004; Reeder and Kramer 2005). The production of glucose provides energy to the organism, helping it return to a homeostatic state (Boonstra et al. 1998; Boonstra 2004; Reeder and Kramer 2005). When an animal experiences chronic stress, the continuous circulation of steroid hormones can cause inhibition of growth, muscle wasting, and suppression of the immune and reproductive systems, all of which can affect the animal's ability to survive, reproduce, and respond to disease (Boonstra and Singleton 1993; Cyr et al. 2007; Charbonnel et al. 2008).

Cortisol has been commonly measured in plasma, saliva, urine, and feces; however, manipulation of the animal, diet, and life-history stages can influence measures of stress hormones in these tissues (Davenport et al. 2006). Moreover, these tissues reflect physiological responses that occur over hours to days (point estimates for plasma and saliva, up to 24 hours for urine, and a couple days for feces), providing no indication of long-term stress unless repeatedly sampled over time (Davenport et al. 2006; Macbeth et al. 2010; Ashley et al. 2011). Alternatively, hair incorporates unbound hormones as it grows, thereby reflecting an animal's stress response over weeks to months (Davenport et al. 2006; Macbeth et al. 2010; Ashley et al.

2011). Hair can also be collected non-invasively and cortisol can remain detectable in hair over years (Webb et al. 2010).

Only recently have studies measured cortisol concentrations in hair to evaluate the effects of disturbance on wildlife. Indeed, Bechshoft et al. (2013) found a strong positive correlation between hair cortisol concentrations and fluctuations in climate and sea ice cover in East Greenland polar bears (*Ursus maritimus*). Moreover, Bryan et al. (2013) found that grizzly bears (*Ursus arctos*) had higher cortisol concentrations following salmon (*Oncorhynchus* spp.) declines, and Bryan et al. (2015) found that wolves had higher cortisol concentrations following increased harvest pressure. To my knowledge, no study has used hair to examine the chronic stress response of boreal woodland caribou or moose to disturbance, and while one study has used hair to examine the effects of harvest on wolf cortisol concentrations (Bryan et al. 2015), no study has used hair to examine wolf stress response to winter severity and diet variability.

Thus, my main objective was to measure cortisol concentrations in hair to determine the chronic stress response of boreal woodland caribou, moose, and wolves to disturbance in eastern Manitoba. Specifically, Chapter 1 examines the stress response of boreal woodland caribou to anthropogenic disturbance while controlling for caribou populations and suitable habitat; Chapter 2 examines the stress response of moose to anthropogenic disturbance and examines the confounding effects of sex, year, latitude, and sample source (i.e., wolf-killed or human-collected) on moose stress response; and Chapter 3 examines the stress response of wolves to increased harvest pressure, winter severity, and diet. I conclude with a final chapter that provides a summary of results, and discussion of management strategies for boreal woodland caribou, moose, and wolves.

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Chapter 1: Stress response of boreal woodland caribou to disturbance in eastern Manitoba

Abstract

Disturbance by anthropogenic activities may cause chronic (long-term) stress in wildlife and contribute to population declines. Declines of boreal woodland caribou (*Rangifer tarandus caribou*; hereinafter referred as caribou) across Canada have been attributed to anthropogenic disturbance, but the physiological stress response of caribou to disturbance has not been examined. To understand the stress response of caribou to disturbance, we measured cortisol concentrations in guard hair collected from 89 female caribou between 2009 and 2011 in eastern Manitoba. We compared cortisol concentrations among five caribou populations occupying areas with varying levels of disturbance, and examined annual variation in cortisol concentrations among the three most southern populations. We also estimated home range sizes of 55 GPS-collared caribou and examined how home range size varied with cortisol concentrations. Within each home range, we quantified disturbance characteristics (roads, transmission lines, cottages, and logged and burned areas) and landscape features (lakes, black spruce, and jack pine habitat), and used model selection to determine the combination of disturbance features that best explained variation in caribou cortisol concentrations. Cortisol concentrations were greatest for the three most southern caribou populations and were higher in 2009 compared to 2011 for these populations. Furthermore, cortisol concentrations increased with decreasing home range size, and proportion of intermediate logging (6 – 21 years) best explained variation in cortisol concentrations. These results suggest that caribou respond negatively to intermediate logging, and constraints on home range size can elevate cortisol concentrations. Since increased cortisol concentrations can affect survival and reproduction, continued monitoring of caribou cortisol

concentrations could be used to manage populations and determine their response to changes in disturbance, climate, and mitigation measures.

Introduction

Anthropogenic disturbances, such as mining, forestry, and energy development, are altering natural ecosystems and contributing to species loss globally (Venter et al. 2006; Fischer and Lindenmayer 2007). In some areas of North America, anthropogenic disturbances have exceeded natural disturbances (Cyr et al. 2009), and habitat loss and fragmentation are considered to be primary factors affecting wildlife within the boreal forest (Venter et al. 2006; Fischer and Lindenmayer 2007; Hins et al. 2009). In particular, conversion of mature forest stands into early-seral stages reduces forest connectivity (Fahrig and Rytwinski 2009), causing wildlife to concentrate into smaller areas or move/expand their ranges in search of more suitable habitat and resources (Andreassen et al. 1998; Selonen et al. 2001; Courtois et al. 2007; Beauchesne et al. 2014).

The mechanisms underlying the effects of disturbance on wildlife are not well known (Macbeth et al. 2010), but studies have recognized that disturbances can provoke a stress response in wildlife and chronic stress may contribute to population declines (Boonstra and Singleton 1993; Wasser et al. 1997; Boonstra et al. 1998; Creel et al. 2002; Wikelski and Cooke 2006; Charbonnel et al. 2008). When an animal is exposed to a stressor, the hypothalamic-pituitary-adrenal axis releases glucocorticosteroids, such as cortisol, into the blood (Boonstra and Singleton 1993; Boonstra 2004; Reeder and Kramer 2005). The release of cortisol triggers gluconeogenesis, the breakdown of glycogen, fats, and proteins to produce glucose, providing energy and helping the organism return to a homeostatic state (Boonstra and Singleton 1993; Boonstra 2004; Reeder and Kramer 2005). When an animal experiences chronic stress, elevated

circulation of cortisol affects its ability to survive, reproduce, and respond to disease (Boonstra and Singleton 1993; Creel et al. 2002; Reeder and Kramer 2005; Charbonnel et al. 2008). Cortisol concentrations have been commonly measured in plasma, saliva, urine, and feces (Davenport et al. 2006; Macbeth et al. 2010; Ashley et al. 2011); however these tissues provide only short-term measures of stress (minutes to days) and stress can be confounded by time of day, manipulation of the animal, environmental disturbance prior to sample collection, and cross-contamination among samples (Davenport et al. 2006). Since circulating unbound cortisol becomes integrated into the hair as it grows, hair provides an indication of chronic stress (weeks to months; Davenport et al. 2006; Macbeth et al. 2010; Ashley et al. 2011).

Boreal woodland caribou (*Rangifer tarandus caribou*; hereinafter referred to as caribou) are listed as threatened under the Manitoba Endangered Species and Ecosystems Act (Manitoba Boreal Woodland Caribou Management Committee 2015). Populations are at risk of decline due to a number of factors, including habitat loss and fragmentation from industrial and fire disturbances, predation, parasites and disease, recreational activities, First Nation harvest, illegal harvest, and climate change (Manitoba Boreal Woodland Caribou Management Committee 2015).

Predation is considered the primary limiting factor of caribou populations in Manitoba (Manitoba Boreal Woodland Caribou Management Committee 2015); however, no studies have directly investigated causes of caribou mortality in Manitoba. Caribou will attempt to minimize predation risk by occupying mature coniferous forest stands and treed muskeg complexes where moose (*Alces alces*) and deer (*Odocoileus virginianus*) densities are low (Bergerud 1985; Bradshaw et al. 1995; Stuart-Smith et al. 1997). They will also separate themselves from other ungulates and predators by living in small groups and by distancing themselves from other

caribou during calving season (Stuart-Smith et al. 1997; Brown et al. 2000; Dyer et al. 2001). Females and calves will also seek lakeshores and islands for refuge from predators in summer (Bergerud 1985; Carr et al. 2011).

Disturbances, such as logging and road development, can alter the landscape and increase caribou interactions with other ungulates, predators, and human hunters (Seip 1991; James and Stuart-Smith 2000; Dyer et al. 2001; Johnson et al. 2015). In particular, linear corridors can increase wolf (*Canis lupus*) and hunter access to caribou habitat (James and Stuart-Smith 2000). Disturbances that open forests can also promote the growth of early successional vegetation and the colonization of deciduous species, which attract moose and deer (Hins et al. 2009), and can subsequently cause an increase in wolf numbers and their predation on caribou (Bergerud and Elliot 1986; Seip 1992; James et al. 2004). Greater interactions between caribou and other ungulate species due to anthropogenic changes in landscape could also lead to an increase in parasite transmission. The meningeal worm (*Parelaphostrongylus tenuis*) is a common nematode parasite that spreads from deer to caribou, causing death in caribou (Anderson 1972; Trainer 1973).

Our objective was to determine the effects of disturbance on the stress response of boreal woodland caribou in eastern Manitoba. While it is known that industrial activity affects the distribution and habitat use of free-ranging boreal woodland caribou (Dyer et al. 2001; Schaefer 2003; Schindler et al. 2007; Courbin et al. 2009; Beauchesne et al. 2014), no studies have examined their stress response to disturbance. We hypothesized that noises, lights, human activity, and traffic resulting from anthropogenic disturbance (i.e., roads, transmission lines, cottages, and logging development) would directly affect caribou. We also hypothesized that anthropogenic (i.e., roads, transmission lines, cottages, and logged areas) and natural

disturbances (i.e., burned areas) would indirectly affect caribou by increasing their interactions with other ungulates, predators, and hunters. Furthermore, we hypothesized that caribou would have smaller home ranges when disturbances impeded their movements (Vors et al. 2007; Beauchesne et al. 2014). Under these hypotheses, which are not mutually exclusive, we predicted cortisol concentrations would increase with increased disturbance within their home range.

Methods

Study area

Our study area (between 50°34' and 53°44' N, and 93°38' and 97°29' W) consists of five caribou populations distributed along the eastern side of Lake Winnipeg, Manitoba: Owl-Flintstone, Atiko, Bloodvein, Berens, and Charron Lake (Figure 1.1). Each population consists of a group of caribou that occupies a distinct area spatially separated from other caribou (Manitoba Boreal Woodland Caribou Management Committee 2015). The study area falls within the Boreal Shield Ecozone, which is characterized by short, warm summers and long, cold winters (Smith et al. 1998). Mean annual temperatures range from 0.3 °C to 1.1 °C, and annual precipitation varies from approximately 540 mm in the northwest to 580 mm in the southeast (Smith et al. 1998). Black spruce (*Picea mariana*) is the dominant tree species, and is found in poorly drained upland and peatland sites, whereas jack pine (*Pinus banksiana*) and trembling aspen (*Populus tremuloides*) are common on upland sites (Smith et al.1998). In well-drained areas, white spruce (*Picea glauca*), balsam fir (*Abies balsamea*), trembling aspen, and balsam poplar (*Populus balsamifera*) can form mixed stands (Smith et al. 1998).

Based on aerial surveys conducted by Manitoba Conservation and Water Stewardship, there are approximately 70 - 75 caribou in the Owl-Flintstone population, 75 - 100 caribou in the Atiko population, 60 caribou in the Bloodvein population, and over 100 caribou in both the

Berens and Charron Lake populations (Dennis Brannen, Caribou Biologist, Manitoba Conservation and Water Stewardship, unpubl. data). The populations primarily occupy treed muskeg habitat and coniferous stands of black spruce and mature jack pine (Schindler 2006; Manitoba Boreal Woodland Caribou Management Committee 2015). Moose, wolves, and black bears (*Ursus americanus*) occupy all caribou ranges (geographic area of suitable area occupied by a caribou population over the last 10 years; Manitoba Boreal Woodland Caribou Management Committee 2015) and some pockets of deer overlap with Owl-Flintstone caribou. No caribou hunting is permitted (The Manitoba Wildlife Act) within game hunting area (GHA) 26, but aboriginal harvesting can occur within other caribou ranges.

Intensity of industrial activity increases from north to south (Figure 1.1). Parts of the Owl-Flintstone range are found within Nopiming Provincial Park, where little industrial activities occur; however, the western portions of the range are vulnerable to industrial activity and development. A large portion of the Atiko range is within Atikaki Provincial Park, and some portions of the Bloodvein and Berens ranges are also within the park. No forestry or mining occurs within the park. Logging operations that had been concentrated around the Owl-Flintstone range and parts of the Bloodvein and Atiko ranges were terminated in September 2009. There was also some historical logging (1984 - 2001) east of the Berens River; otherwise, fire is the main source of disturbance within the two northern caribou ranges. Some mineral exploration occurs within the southern caribou ranges (Owl-Flintstone, Atiko, and Bloodvein), but exploration is transient and there are no operational mine sites within these areas. Mineral exploration and drill sites could have increasing impact through construction of access roads and trails, so we focused on examining the impacts of roads and trails on caribou rather than drill sites.

Telemetry and disturbance data

We collected hair from 89 female caribou that were captured and collared with global positioning system (GPS) collars (Lotek 3300L and Iridium models) programmed to transmit locations every 1 or 3 hours. Hair was collected in January/February 2010 and 2012 for the three southern caribou populations (Owl-Flintstone, Atiko, and Bloodvein), and January/February 2011 for the two northern caribou populations (Berens and Charron Lake). Since cortisol becomes integrated into the hair as it grows, cortisol results would reflect caribou physiology the summer and fall (approximately June 1st - October 31st) prior to hair collection: summer-fall 2009 and 2011 for the southern populations, and summer-fall 2010 for the northern populations. We used telemetry data from the previous summer-fall for individuals that were re-captured, but for newly captured individuals, we used telemetry data recorded the summer-fall following hair collection. Since boreal woodland caribou show strong site fidelity and follow the same migration route year to year (Schaefer et al. 2000; Metsaranta 2007), we were confident that caribou would have experienced similar habitat disturbances over a two year period. In fact, home range overlap of 20 individual caribou over two consecutive years averaged 73% (± 26% SD) and home range size did not differ between years (paired t-test: $t_{19} = 1.00$; $p = 0.33$).

We used ArcGIS 10.2 and Geospatial Modelling Environment to create 100% minimum convex polygons (MCPs) to encompass caribou movements in summer-fall. We examined movements of 55 of the 89 caribou with complete telemetry recordings between June 1st and October 31st that did not venture into Ontario, where access to habitat data was limited. We used MCPs over kernel density estimates because MCPs allowed us to quantify disturbances that might not have directly interfered with caribou, but were still recognized by caribou (e.g., loud noises, logged habitat, etc.). Indeed, we added a 5 km-radius buffer around each caribou's MCP

to account for all surrounding disturbances that could have affected caribou stress response (Leblond et al. 2012).

We determined the area of each caribou's MCP and quantified disturbances and habitat features obtained from the Manitoba Land Initiative (http://mli2.gov.mb.ca/) or Manitoba Conservation and Water Stewardship. We quantified road, transmission line, and cottage densities within caribou ranges. We divided roads into two categories based on their traffic intensity: major roads (highways, provincial, municipal, community, and long-term all-weather class 1 and 2 roads) and minor roads (trails, forestry, mining, park, and short-term all-weather class 3 and 4 roads). We also determined the proportion of recent (0 - 5 years), intermediate (6 – 21 years), and old (22 – 41 years) logging and fire disturbances prior to the time individuals were collared. When logging and fire areas overlapped, we selected the most recent disturbance (e.g., selected logging 5 years ago over fire 35 years ago).

We also quantified habitat features known to provide important refuge to caribou. We determined the proportion of lakes, black spruce and tamarack larch muskeg, black spruce forest, and mature jack pine forest. Black spruce stands included 71 - 100% black spruce, and 40 - 70% black spruce with jack pine, white spruce, balsam fir, or tamarack as secondary species (Schindler 2006). Jack pine stands included jack pine trees > 50 years with 71 - 100% cover, and 40 - 70% cover with black spruce, balsam fir, white spruce, and tamarack as secondary species (Schindler 2006). Caribou avoid areas up to 50 years following logging and fire disturbances (Joly et al. 2003; Metsaranta and Mallory 2007), so we did not include muskeg and forest stands that were logged and/or burned up to 50 years from when caribou were collared.

Cortisol analysis

We separated guard hair from underfur in our samples because guard hair would have grown throughout summer-fall, which represents a vulnerable time for female caribou and their newborn calves. Furthermore, guard hair was easier to clean and homogenize and produced less variable cortisol concentrations among and within body regions compared to underfur (Macbeth et al. 2010). We removed surface contaminants from hair by performing two 3-min methanol washes (0.1 mL of methanol/mg hair) per sample (Appendix A). We then dried samples under a fume hood for at least two days and ground the hair into a fine powder for 0.03 min/mg hair at 30 Hz using a ball mill (Retsch MM 301 Mixer Mill, Retsch Inc, Newtown, Pennsylvania, USA). For samples that did not powder sufficiently, we continued to grind the hair at 30 Hz until it reached a powder consistency similar to other samples (Macbeth et al. 2010). We then added 1 mL of methanol per 50 mg hair and placed the samples on a shaker table (Standard Orbital Shaker Model 3500, VWR®) for 24 hours, then centrifuged the samples (15 min at 4500 rpm, at 20°C) and collected the supernatant into polypropylene tubes. To ensure cortisol was extracted sufficiently, we rinsed the samples with 1 mL of methanol, vortexed them (30 sec), centrifuged them for another 15 minutes (4500 rpm at 20°C), and pooled the supernatants. We repeated this process two times for a total of three collections per sample. We then dried the pooled supernatant in a sample concentrator (Savant® ISS110 SpeedVac® Concentrator) and reconstituted samples with 0.25 – 0.5 mL radioimmunoassay (RIA) buffer on the day of the assay (0.1 M phosphate buffer, 0.9% w/v NaCl, and 0.5% w/v bovine serum albumin).

We used RIA techniques as described by Ryan et al. (2012) to measure cortisol concentrations in hair samples. In polypropylene tubes, we combined 100 µL of cortisol-specific antibody (1: 9000 dilution; Fitzgerald Industries, Acton, MA, USA, product code 20-CR50) with 100 µL of 5000 disintegrations per minute (dpm) of tritiated cortisol tracer (Perkin Elmer,

Waltham, MA, USA) and 100 µL of either reconstituted sample or known cortisol concentrations (standards). Cross-reactivity of the antibody used was 100% for cortisol, 5.7% for 11-deoxycortisol, 3.3% for corticosterone, 36% for prednisolone, and < 0.7% for cortisone. We let the samples incubate for one hour at room temperature and then overnight at 4°C. The following day, we added 100 µL of dextran (0.5% w/v)-coated charcoal (5% w/v), briefly vortexed the samples, and let them incubate for 15 min on ice. We then centrifuged the samples for 30 min (2500 g-force at 4°C), collected the supernatant into 6 mL scintillation vials, added 4 mL of Ultima Gold scintillation fluid (Perkin Elmer), and determined radioactivity using a scintillation counter (TriCarb 3100 LSC, Perkin Elmer, Waltham, MA, USA). Using the known cortisol concentrations of a standard curve (13 concentrations ranging from 0.013 to 50 ng/mL), we interpolated cortisol concentrations for each sample. To increase the accuracy of our results, we processed all samples in duplicate and standards in triplicate. Extraction efficiency for pooled caribou hair was 70.87% ± 3.89% (Mean ± SE) based on five recoveries of 0.7 ng cortisol/mL methanol of spiked extract from ground hair. Cortisol concentrations from serial-diluted pooled caribou hair were parallel with serially diluted standard concentrations ($R^2 = 0.94$; $p = 0.029$). Intra-assay coefficient of variability (n = 6) was 9.07% and the inter-assay coefficient of variability (n = 8) was 18.90%.

Data analysis

To determine whether cortisol concentrations (log-transformed) differed among caribou populations, we used analysis of variance (ANOVA) followed by a post-hoc Tukey test. We included year nested within individual as a random effect since several of the same caribou within the Owl-Flintstone range were measured in 2009 and 2011. We also recognized that annual variability could have affected caribou cortisol concentrations, so we determined whether

cortisol concentrations (log-transformed) differed between year (2009 and 2011) and population (Owl-Flintstone, Atiko, and Bloodvein ranges) using a two-way ANOVA with an interaction and including individual as a random effect. We did not include the two northern populations in this analysis because we only had cortisol data for those populations from a single year (2010), which differed from the two years of sample collection for the three southern populations. Finally, we grouped caribou by population (n = 5) and used linear regression to examine how mean home range size related with mean cortisol concentrations (log-transformed).

Prior to determining the effects of disturbance on caribou cortisol concentrations, we standardized all variables to a mean of zero and a standard deviation of one. We then determined a priori models (n = 29) based on combinations of disturbance variables suspected to have the greatest effects on caribou. Based on previous studies, we focused on creating models with combinations of anthropogenic disturbances, such as logging and corridors (Vors et al. 2007; Beauchesne et al. 2014), and included interactions between logging and roads, cottages and roads, and cottages and transmission lines. Due to high multicollinearity ($|r| > 0.7$), we did not include major roads with minor roads, logging (recent, intermediate, and old), or cottages; recent logging with old logging or cottages; or minor roads with intermediate logging.

We compared a series of linear mixed models to determine which models best predicted cortisol concentrations (log-transformed) in caribou. Since our caribou populations occurred across a north-south gradient, we included population as a random effect in each model. We also included black spruce, jack pine, and lakes as caribou habitat covariates in each model, but did not include treed muskeg since it was highly correlated with jack pine ($r = -0.76$). We then ranked the candidate models based on Akaike's information criterion corrected for small sample sizes (AICc), and calculated the Akaike weight (the relative information content) for each model.

Models with $\Delta AIC_c < 2$ were considered good models (Burnham and Anderson 2002), and variables were considered to be informative when their 95% confidence intervals (CI) did not encompass zero (Arnold 2010). Finally, we determined the relative importance of each variable by summing the weights of each candidate model containing that variable. All analyses were completed using JMP® Version 12 (SAS Institute Inc., Cary, North Carolina, USA) using a significance level of $\alpha = 0.05$.

Results

Hair cortisol concentrations differed among caribou populations ($F_{4,84} = 6.19$; $p = 0.0002$; $n = 89$; Figure 1.2). In particular, cortisol concentrations were lower in Berens caribou than Owl-Flintstone (Tukey's HSD, $p = 0.025$), Atiko ($p = 0.016$), and Bloodvein ($p = 0.002$) caribou, and lower in Charron Lake caribou than Bloodvein caribou ($p = 0.047$). Caribou cortisol concentrations were greater in 2009 than 2011 ($F_{1,28} = 8.05$; $p = 0.008$; $n = 34$; Figure 1.3). Specifically, Bloodvein caribou cortisol concentrations were greater in 2009 than 2011 (Tukey's HSD, $p = 0.042$). We found no main effect for population ($F_{2,26} = 2.39$; $p = 0.111$), but there was an interaction between year and population ($F_{2,26} = 4.36$; $p = 0.023$). Finally, caribou cortisol concentrations increased with decreasing mean home range size ($R^2 = 0.798$; $F_{1,3} = 11.83$; $p = 0.041$; $n = 5$; Figure 1.4).

Disturbances varied among the five caribou populations (Table 2.1). The model with intermediate logging provided the best explanation of caribou cortisol levels (Model 1: $R^2 = 0.357$; Table 1.2); cortisol concentrations increased with increasing proportion of intermediate logging within home range and buffer areas. As the 95% CI of the model coefficient for intermediate logging did not encompass zero ($\beta = 0.693 \pm 0.579$), this variable was considered informative. The habitat features black spruce, jack pine, and lakes were uninformative in our

top model because the 95% CIs of their model coefficients did encompass zero (-0.269 ± 0.416, -0.280 ± 0.480, and -0.103 ± 0.270, respectively). No other tested models provided good support ($\Delta AIC_c > 2$), and intermediate logging had the greatest relative importance (Table 1.3).

Discussion

Hair cortisol concentrations were greater for the three southern caribou populations, where anthropogenic disturbances were generally high. In particular, these caribou experienced the greatest amount of recent and intermediate logging within their ranges. Cortisol concentrations were also greater for the three southern populations in 2009 compared to 2011; however, the annual variation we observed in cortisol concentrations was driven primarily by the Bloodvein population, which had small sample sizes in both 2009 (n = 2) and 2011 (n = 3). Consequently, it is difficult to discern how much annual variability in fact affects caribou cortisol concentrations in our study area.

We also found that caribou cortisol concentrations increased with decreasing home range sizes. In particular, home ranges were smaller and cortisol concentrations were higher for the three southern caribou populations compared to the two northern caribou populations. Disturbances can reduce connectivity within the landscape (Fahrig and Rytwinski 2009) and reduce habitat availability and quality (Eigenbrod et al. 2008). For migratory species such as caribou, individuals typically expand their ranges to avoid areas with disturbance; however, when movements are restricted by disturbances, individuals might constrict their ranges (Smith et al. 2000; Courtois et al. 2007; Beauchesne et al. 2014). Indeed, Smith et al. (2000) found that caribou home range size and movements decreased following logging. Courtois et al. (2007) also found that caribou expanded their home ranges when fire and logging disturbances increased from 0 -40%, but contracted their ranges when these disturbances exceeded 40%. We never had

greater than 40% fire and logging disturbances within caribou home ranges, but other disturbances in combination with fire and logging, such as roads, transmission lines, and cottages, could have contributed to total disturbance across the landscape and contraction of caribou home ranges in the south.

Natural and anthropogenic disturbances also had differential effects on caribou. Fire can negatively affect caribou habitat by reducing lichen abundance (Dyer et al. 2001; Faille et al. 2010), but caribou have evolved in habitat affected by natural disturbances and are not limited by lichen if other suitable forage is available following fire disturbances (Dyer et al. 2001). Anthropogenic disturbances can impose irreversible damage to habitat (Dyer et al. 2001), and affect caribou to a greater extent than natural disturbances (Dalerum et al. 2007; Vors et al. 2007; Faille et al. 2010; Beauchesne et al. 2014). Our results suggested that fire was not a good predictor of caribou cortisol concentrations compared to anthropogenic disturbances. Indeed, intermediate logging had the greatest effect on caribou cortisol concentrations.

Logging is considered to be the main form of anthropogenic disturbance within the boreal forest (Burton et al. 1999). It reduces connectivity and affects the age and quality of natural forest stands by converting old growth forest into early-seral stages favoured by moose, deer, wolves, and black bears (Bergerud and Elliot 1986; Seip 1992; Wittmer et al. 2005). Studies have found that logging is the primary disturbance affecting caribou distribution (Mahoney and Virgl 2003; Schaefer 2003; Schaefer and Mahoney 2007; Vors et al. 2007; Hins et al. 2009). In particular, Hins et al. (2009) found that caribou selected lichen woodland and peatlands during calving, but avoided logged areas between 6 and 20 years. Moreover, Vors et al. (2007) found that forest cutovers, particularly recent cutovers (< 10 years) were the best predictors of caribou

decline. Johnson et al. (2015) also found that caribou subpopulations occurred > 3 km away from logged areas during summer.

Logging is often associated with dense road networks that fragment habitat and impede caribou movements (Dyer et al. 2002). Beauchesne et al. (2014) found that logging and roads combined affected space use of caribou. Moreover, James and Stuart-Smith (2000) found that caribou kills by wolves were greatest around corridors (roads, transmission lines, and pipelines). Roads increase wolf access to caribou by improving their visibility through the forest, increasing their ability to smell and track down prey, enhancing their speed through peatland habitat, and altering their movements so that they expand their ranges into caribou habitat (Latham et al. 2011a). Although major and minor roads were not informative variables in our models, they were highly and positively correlated with logging, so they could have affected caribou in combination with logging.

Transmission lines can also alter caribou distribution (Nellemann et al. 2001) and provide wolves with greater access to caribou habitat (i.e., linear corridors). Nonetheless, transmission lines were not a good predictor of caribou cortisol levels in our models. Cottages were also not a good predictor of caribou cortisol concentrations despite other studies finding that cottages can disturb caribou and force them to shift their ranges (Nellemann et al. 2010; Polfus et al. 2011). Cottages were mainly found within the Owl-Flintstone range, but otherwise were not very abundant within other caribou ranges. Moreover, cottages might have been abandoned or might not have been active throughout the year; thus, reducing their impact on caribou.

The effects of disturbance on caribou can be exacerbated if suitable habitat is low (Stuart-Smith et al. 1997; Schindler 2006). Although, black spruce, jack pine, and lakes were not informative variables in our top model, they are still important features that can affect caribou

habitat use and distribution (Schindler 2006). Caribou will attempt to minimize spatial overlap with other ungulates and predators by occupying mature stands of coniferous forest and treed muskeg habitat (Bradshaw et al. 1995; Stuart-Smith et al. 1997; Schindler 2006). In particular, Schindler (2006) found that caribou in the Owl-Flintstone and Bloodvein ranges selected for jack pine, black spruce, and treed muskeg even when these habitat types were not abundant within the landscape. Females will also attempt to separate themselves from other ungulates and predators by using lakeshores and islands for refuge in summer (Bergerud 1985). A loss of suitable habitat could increase caribou movements as they search for other suitable habitat or cause them to crowd into smaller areas, increasing their vulnerability to predators, hunters, and parasite and disease transmission from other ungulates (Dyer et al. 2001).

Conclusions

Our results suggest that disturbance in the southern part of our study area could be causing caribou to contract their ranges, thereby increasing their cortisol concentrations. Additional industrial development could further constrict caribou ranges, eventually causing population level consequences, including reduced reproductive success and greater mortality (Beauchesne et al. 2014). Reducing anthropogenic development in areas where caribou home ranges are already small or constricting could help prevent population declines. In particular, restricting logging activities from caribou ranges could reduce habitat loss and fragmentation. Protecting large forest blocks of mature coniferous forest and treed muskeg habitat (100 - 250 km^2; Courtois et al. 2007) would reduce caribou interactions with competitors, predators, and humans. In the event that logging occurs, grouped together cutblocks and associated roads could reduce further fragmentation of old successional forest (Hins et al. 2009).

Although logging explained much of the variation in caribou cortisol concentrations, other factors unaccounted for in this study could have also affected caribou, including predator densities, parasite prevalence, hunting pressure, and weather conditions. Studies suggest that predation is a primary factor affecting caribou (Seip 1992; McLoughlin et al. 2003; James et al. 2004). Wolves are considered the main predator of caribou (Bergerud and Elliott 1986; Rettie and Messier 1998; James et al. 2004), but black bears also target caribou neonates (typically first 4 - 6 weeks; Bergerud 1971; Latham et al, 2011b). Habitat use by black bears can be variable, with some bears occupying peatland and bogs where caribou are found (Latham et al. 2011b), so management of both wolf and black bear populations to natural low densities may be important for preventing further caribou declines. Deer overlap with caribou in the Owl-Flintstone range can also result in parasite transmission, including transmission of the meningeal worm, which is detrimental to caribou (Anderson 1972; Trainer 1973). Minimizing anthropogenic development could reduce transmission of disease and parasites between deer and caribou in the south. Future studies should also consider the consequences of climatic variation on caribou, particularly since climate warming could result in extreme and unpredictable weather events, earlier green-up, and greater insect emergence (Vors and Boyce 2009).

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Table 1.1. Summary (mean ± SD) of home range sizes with 5 km-radius buffers, disturbances, habitat features, and hair cortisol concentrations for five boreal woodland caribou (*Rangifer tarandus caribou*) populations in eastern Manitoba.

	Owl-Flintstone (n = 7)	Atiko (n = 5)	Bloodvein (n = 3)	Berens (n = 28)	Charron Lake (n = 12)
Home range size (km^2)	860 ± 220	780 ± 170	420 ± 130	1,460 ± 670	1,250 ± 640
Roads $_{major}$ (m/km^2)	70 ± 10	0 ± 0	50 ± 10	0.05 ± 0.3	0 ± 0
Roads $_{minor}$ (m/km^2)	200 ± 60	30 ± 40	200 ± 20	80 ± 40	20 ± 20
Transmission lines (m/km^2)	20 ± 10	0 ± 0	40 ± 10	30 ± 10	0 ± 0
Cottages ($no./km^2$)	0.01 ± 0.01	0.001 ± 0.001	0 ± 0	0.001 ± 0.001	0 ± 0
Logging $_{recent}$ (%)	1 ± 0.4	0.08 ± 0.1	0.02 ± 0.04	0 ± 0	0 ± 0
Logging $_{intermediate}$ (%)	2 ± 0.3	0.1 ± 0.1	4 ± 0.9	0.09 ± 0.2	0 ± 0
Logging $_{old}$ (%)	4 ± 3	0.03 ± 0.07	0.005 ± 0.01	0.2 ± 0.3	0 ± 0
Fire $_{recent}$ (%)	0.004 ± 0.0004	0.002 ± 0.002	0.4 ± 0.7	1 ± 4	0.5 ± 0.9
Fire $_{intermediate}$ (%)	0.05 ± 0.01	1 ± 3	2 ± 0.5	1 ± 3	5 ± 8
Fire $_{old}$ (%)	15 ± 5	13 ± 3	9 ± 3	14 ± 9	6 ± 7
Lakes (%)	7 ± 2	4 ± 5	3 ± 1	3 ± 4	4 ± 3
Black spruce (*Picea mariana*; %)	10 ± 0.7	5 ± 0.5	17 ± 2	5 ± 2	9 ± 4
Jack pine (*Pinus banksiana*; %)	30 ± 2	30 ± 4	10 ± 1	18 ± 7	5 ± 2
Treed muskeg (%)	12 ± 1	20 ± 6	22 ± 0.9	27 ± 10	44 ± 11
Cortisol concentrations (ng/g)	3.38 ± 3.28	4.10 ± 4.30	7.19 ± 6.10	1.71 ± 0.61	2.02 ± 0.59

Table 1.2. Comparison of generalized linear mixed models* with disturbances explaining boreal woodland caribou (*Rangifer tarandus caribou*) cortisol concentrations (log-transformed) based on Akaike's information criterion corrected for small sample sizes (AICc). The top 20 of 29 models are presented below, including the null† and global models.

Model	Model variables	R^2	-2 LogL	*N*	*K*	AICc	ΔAICc	Relative L	AICc Weight
1	Logging $_{intermediate}$	0.357	148.05	55	7	164.43	0.00	1.00	0.50
2	Logging $_{recent,}$ logging $_{intermediate}$	0.370	147.82	55	8	166.95	2.52	0.28	0.14
3	Null	0.313	153.95	55	6	167.70	3.27	0.20	0.10
4	Logging $_{intermediate}$, logging $_{old}$	0.363	149.01	55	8	168.14	3.71	0.16	0.08
5	Logging $_{recent}$	0.340	153.98	55	7	170.36	5.93	0.05	0.03
6	Transmission lines	0.338	154.01	55	7	170.39	5.96	0.05	0.03
7	Roads $_{major}$	0.327	154.20	55	7	170.58	6.15	0.05	0.02
8	Logging $_{old}$	0.334	154.84	55	7	171.22	6.79	0.03	0.02
9	Cottages	0.315	154.89	55	7	171.27	6.84	0.03	0.02
10	Roads $_{minor}$	0.325	154.98	55	7	171.36	6.93	0.03	0.02
11	Fire $_{old}$	0.316	155.45	55	7	171.83	7.40	0.02	0.01
12	Fire $_{recent}$	0.315	155.46	55	7	171.84	7.41	0.02	0.01
13	Fire $_{intermediate}$	0.315	155.67	55	7	172.05	7.62	0.02	0.01
14	Roads $_{major}$, transmission lines	0.360	154.14	55	8	173.27	8.84	0.01	0.01
15	Cottages, transmission lines	0.343	154.76	55	8	173.89	9.46	0.01	0.00
16	Logging $_{recent}$, roads $_{minor}$	0.341	154.82	55	8	173.95	9.52	0.01	0.00
17	Roads $_{minor}$, transmission lines	0.344	155.04	55	8	174.17	9.74	0.01	0.00
18	Cottages, roads $_{major}$	0.330	155.09	55	8	174.22	9.79	0.01	0.00
19	Cottages, roads $_{minor}$	0.327	155.87	55	8	175.00	10.57	0.01	0.00
20	Cottages x transmission lines	0.349	154.16	55	9	176.16	11.73	0.00	0.00
29	Global	0.391	150.99	55	17	164.43	0.00	1.00	0.50

*****Models are summarized by the -2 Loglikelihood (-2 LogL), sample size (*N*), number of parameters (*K*), AICc score, the difference between the AICc for that model and the lowest AICc for any model (ΔAICc), the relative likelihood (Relative L), and AICc weight (model probability).
†Null model consists of caribou population as a random effect and jack pine, black spruce, and lakes as covariates.

Table 1.3. Relative importance (sum model weights) of disturbances based on the models compared in Table 1.2.

Variable	Relative importance
Logging $_{\text{intermediate}}$	0.714
Logging $_{\text{recent}}$	0.171
Logging $_{\text{old}}$	0.094
Transmission lines	0.042
Roads $_{\text{major}}$	0.034
Cottages	0.030
Roads $_{\text{minor}}$	0.028
Fire $_{\text{recent}}$	0.014
Fire $_{\text{intermediate}}$	0.014
Fire $_{\text{old}}$	0.014

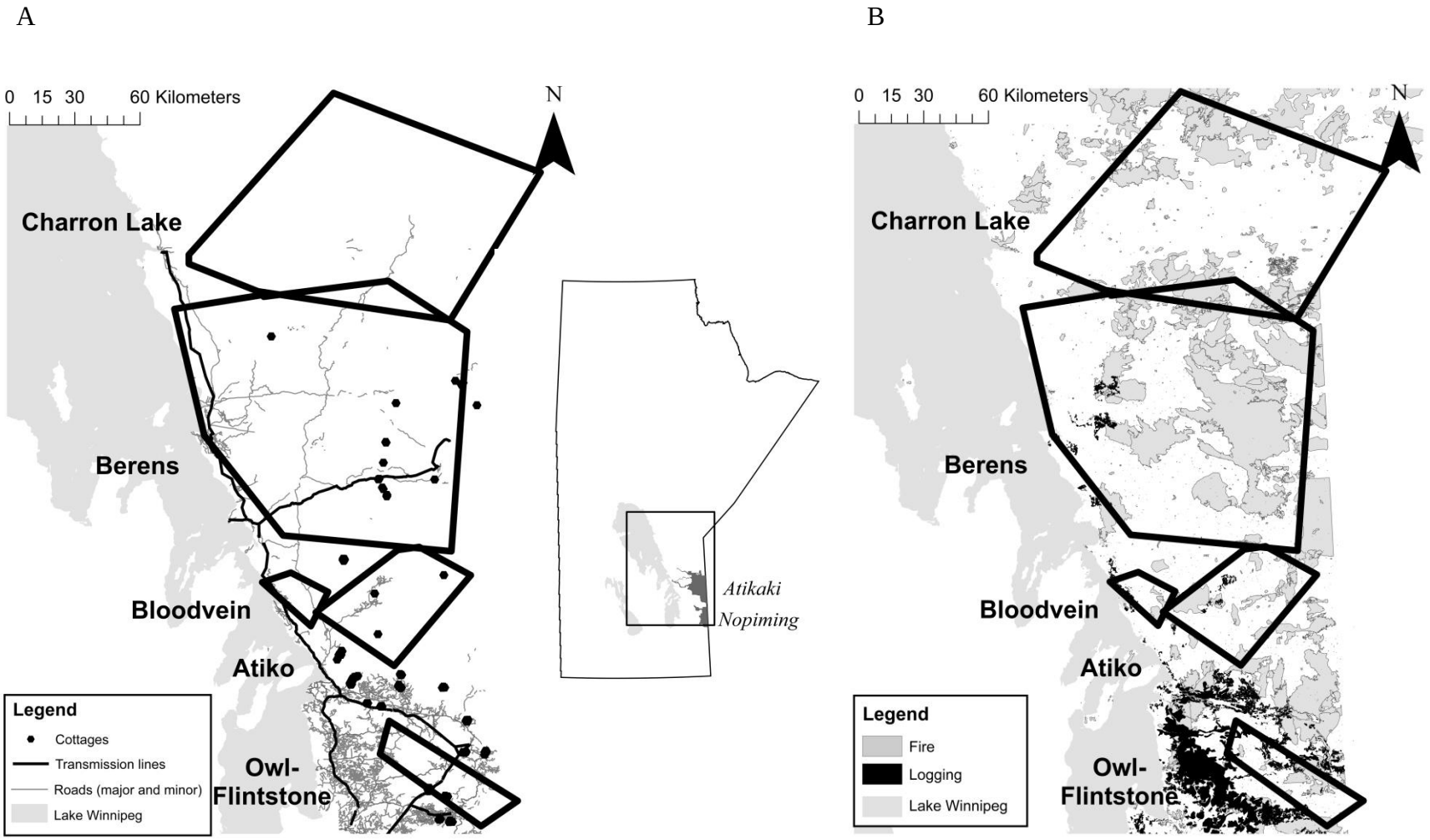

Figure 1.1. Distribution of disturbances surrounding and within the ranges of five boreal woodland caribou (*Rangifer tarandus caribou*) populations (Owl-Flintstone, Atiko, Bloodvein, Berens, and Charron Lake) in eastern Manitoba, including (A) cottages, transmission lines, and roads, and (B) logging (1969 - 2009) and fire (1969 - 2011).

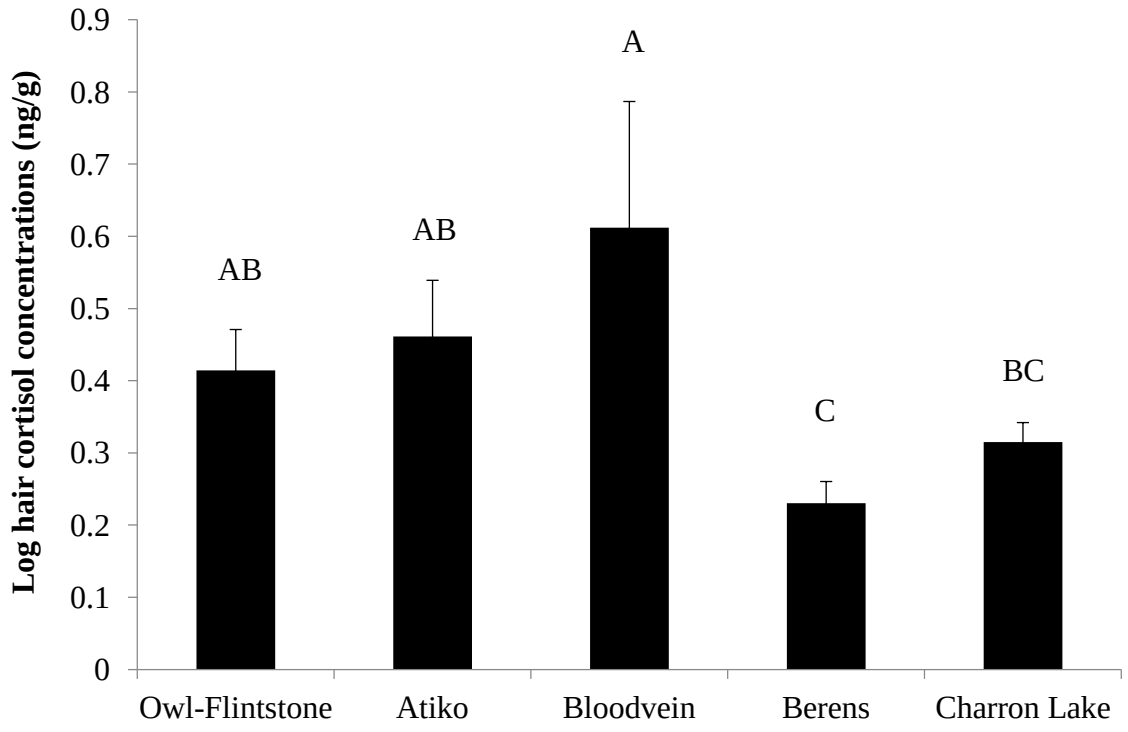

Figure 1.2. Cortisol concentrations (log-transformed, mean ± SE) of Owl-Flintstone (n = 18), Atiko (n = 11), Bloodvein (n = 5), Berens (n = 37), and Charron Lake (n = 18) boreal woodland caribou (*Rangifer tarandus caribou*) populations in eastern Manitoba. Means not sharing the same letter are significantly different.

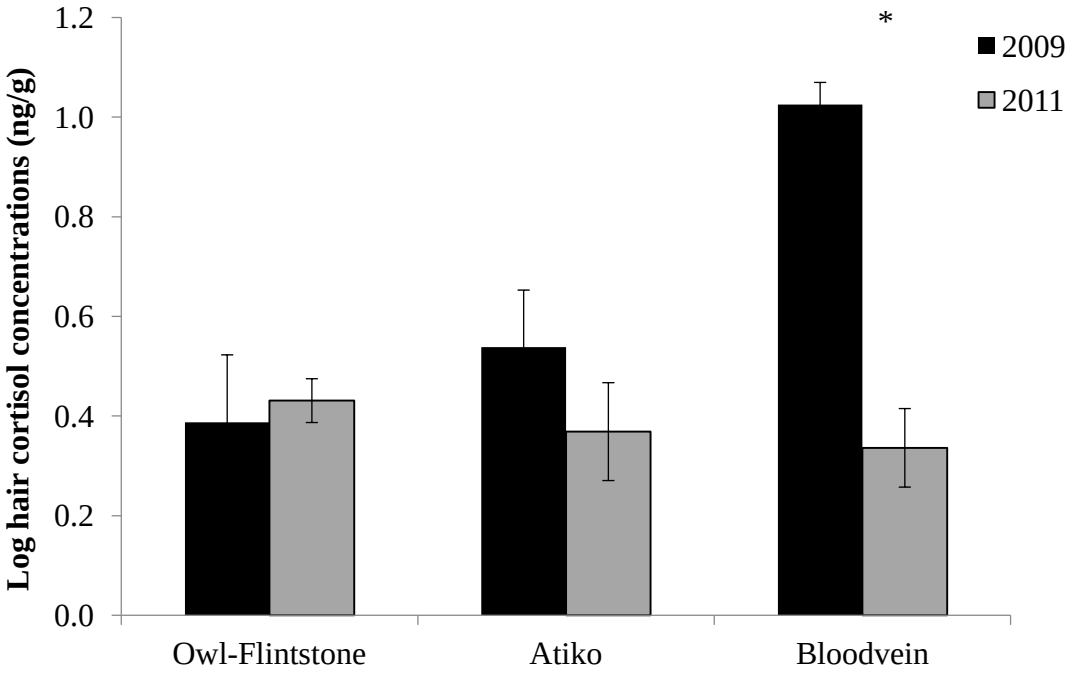

Figure 1.3. Cortisol concentrations (log-transformed, mean ± SE) of Owl-Flintstone (2009: n = 7; 2011: n = 11), Atiko (2009: n = 6; 2011: n = 5), and Bloodvein (2009: n = 2; 2011: n = 3) boreal woodland caribou (*Rangifer tarandus caribou*) populations in 2009 and 2011 in eastern Manitoba. *Significant annual variation within a caribou population.

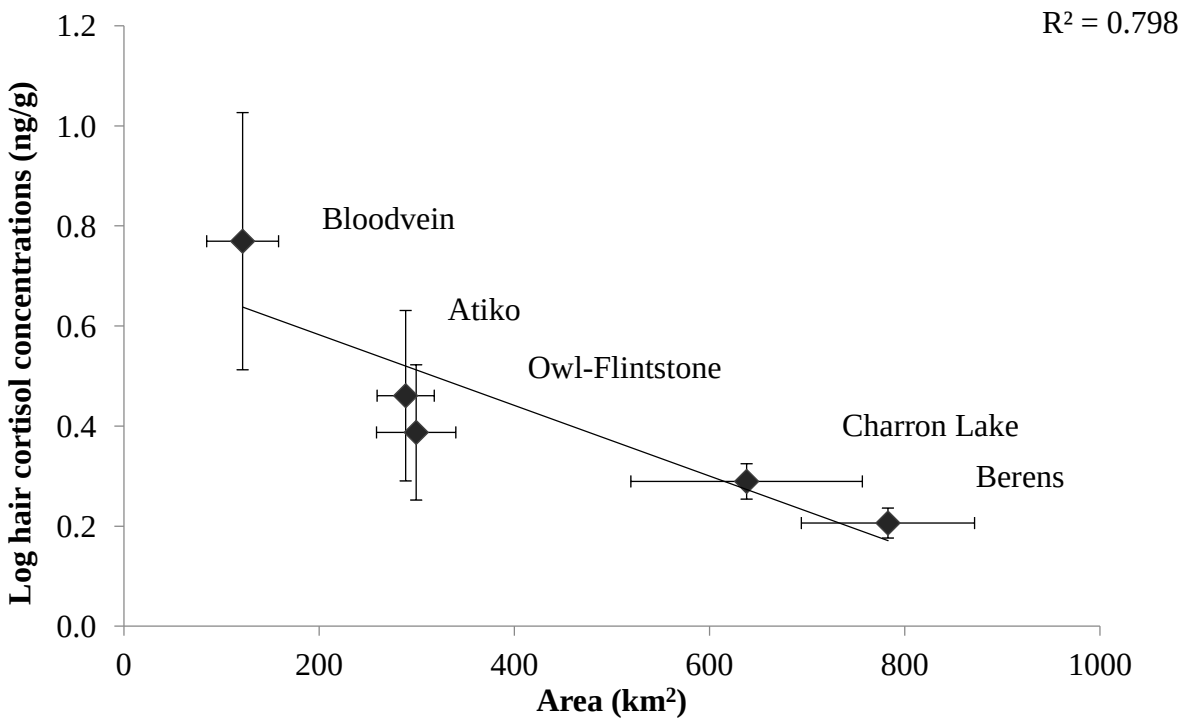

Figure 1.4. Relationship between boreal woodland caribou (*Rangifer tarandus caribou*) home range size (mean ± SE) and cortisol concentrations (log-transformed, mean ± SE) for the Owl-Flintstone (n = 7), Atiko (n = 5), Bloodvein (n = 3), Berens (n = 28), and Charron Lake (n =12) populations.

Chapter 2: Anthropogenic disturbance does not affect chronic stress in eastern Manitoba moose

Abstract

Anthropogenic disturbance can cause chronic stress in wildlife and contribute to population decline. Moose populations declined by 65% between 2000 and 2010 in eastern Manitoba, and disturbance could be an important cause for decline. To determine chronic stress of moose to disturbance, we measured cortisol concentrations of moose guard hair collected between 2010 and 2014 in eastern Manitoba by humans and from wolf kill sites. To evaluate the effects of disturbance on moose cortisol concentrations, we quantified disturbance characteristics (roads, transmission lines, logging, and fire) within a 4.6 km-radius buffer around each moose sample location. We then used model selection to determine the combination of disturbance features that best explained variation in moose cortisol concentrations (n = 63) while controlling for sample source (cortisol concentrations were greater in wolf-killed moose than human-collected moose). Our disturbance variables did not affect moose cortisol concentrations. Sample source was an informative variable in our top model, suggesting that chronic stress in moose, reflected in high cortisol concentrations, increases their vulnerability to wolf predation. Although our disturbance variables had no effect on moose cortisol concentrations, other factors such as parasitism, predation, and harvest pressure could affect moose stress response. Measuring cortisol concentrations in hair could be an important tool for evaluating population health, particularly with changes in management strategies, human disturbance, and climate change.

Introduction

Measures of stress hormones in wildlife have been increasingly used to assess individual health and condition (Wikelski and Cooke 2006). Indeed, studies have demonstrated that anthropogenic disturbance can provoke a stress response in individuals, and chronic (long-term)

stress can contribute to population decline (Boonstra and Singleton 1993; Wasser et al. 1997; Creel et al. 2002; Pride 2005). With increasing habitat loss and fragmentation due to human infrastructure development, it is important to understand the negative consequences of disturbance on wildlife populations (Terwissen et al. 2013).

When an animal encounters a stressor in its environment, the hypothalamic-pituitary-adrenal axis is activated and glucocorticosteroids are released from the adrenal cortex into the blood (Boonstra and Singleton 1993; Boonstra 2004). Glucocorticosteroids assist in recovery of the animal to baseline state through mobilization of energy (Boonstra and Singleton 1993; Boonstra et al. 1998; Boonstra 2004; Reeder and Kramer 2005). Continuous circulation of glucocorticosteroids resulting from chronic environmental stressors can be detrimental and cause muscle wasting, immunosuppression, and inhibition of growth and reproduction (Boonstra and Singleton 1993; Boonstra 2004; Reeder and Kramer 2005). Cortisol is the main stress hormone produced in most mammals and has been commonly measured in plasma, urine, and feces (Davenport et al. 2006; Macbeth et al. 2010; Ashley et al. 2011). Cortisol concentrations from these media can be confounded by short-term stressors, such as feeding and manipulation of the animal (Davenport et al. 2006; Macbeth et al. 2010; Ashley et al. 2011). Cortisol becomes integrated into the hair as it grows, so hair represents an ideal medium for evaluating chronic stress in free-ranging mammals (Davenport et al. 2006; Macbeth et al. 2010; Ashley et al. 2011).

Moose (*Alces alces*) populations in southeastern Manitoba declined by 65% between 2000 and 2010 (from 2,350 to 823 moose based on aerial surveys; Leavesley 2010), and a number of factors could be responsible, including disturbance, parasitism, predation, hunting, reduced habitat quality, and climate change (Gasaway et al. 1992; Kunkel and Pletscher 2000; Murray et al. 2006; Shura and Roth 2013). Areas disturbed by logging and fire can provide

important early successional browse for moose (Monthey 1984; Gasaway et al. 1989; Rempel et al. 1997; Wasser et al. 2011), but major roads could limit moose movements and cause mortality (Laurian et al. 2008; Beyer et al. 2012; Eldegard et al. 2012: Bartzke et al. 2015). Indeed, moose avoid corridors with high traffic intensity, such as major roads (Laurian et al. 2008; Beyer et al. 2012; Bartzke et al. 2015), but may browse early successional vegetation and lick salt pools along corridors with minimal human traffic, such as minor roads, trails, and transmission lines (Grosman et al. 2011; Eldegard et al. 2012; Bartzke et al. 2015). Fragmented habitat can also increase moose interactions with white-tailed deer (*Odocoileus virginianus*), wolves (*Canis lupus*), and hunters. In regions where deer densities are high, moose could contract parasites, such as the meningeal worm (*Parelaphostrongylus tenuis*), which causes mortality in moose (Anderson 1964; Whitlaw and Lankester 1994). Moose are also vulnerable to wolf and black bear (*Ursus americanus*) predation, especially in spring and summer when calves are born (Boutin 1992; Gasaway et al. 1992; Mocker 2015). Furthermore, corridors and fragmented habitat can increase predator and hunter access to moose (Rempel et al. 1997; Kunkel and Pletscher 2000; Crichton et al. 2004; Potvin et al. 2005).

To better understand moose decline, it is important to understand the physiological mechanisms affecting individual health and condition. A number of environmental and intrinsic factors could affect moose physiology, including anthropogenic disturbance, parasitism and disease, predation, hunting, starvation, temporal variation in climate, reduced habitat quality, injury, and variation in sex and age. We focussed our study on investigating chronic stress of moose to disturbance in eastern Manitoba. We hypothesised that human disturbance, such as vehicle traffic and noise, would directly affect moose, and predicted that moose cortisol concentrations would increase with increasing major road densities (i.e., high human traffic) in

the vicinity of the sampling site. We also hypothesised that disturbance would provide important early successional forage for moose, and predicted that cortisol concentrations would decrease with increasing minor roads and transmission line densities, and regenerating logged and burned areas (i.e., disturbance with limited human presence). Alternatively, we hypothesised that disturbance would increase moose interactions with parasites, predators, and hunters, and predicted that moose cortisol concentrations would increase with increasing major road, minor road, and transmission line densities, and logged and burned areas.

Methods

Study area

Moose hair was collected by Manitoba Conservation and Water Stewardship from wolf-killed, hunted, road-killed, and collared individuals from winters (September to April) 2010-11 to 2014-15 in game hunting areas (GHAs) 26, 17, and 17A. The study area is found within the Lac Seul Upland Region of the Boreal Shield Ecozone (between 50°36' and 52°41' N, and 95°9' and 96°37' W). The climate consists of short, warm summers and long, cold winters with mean annual temperatures ranging between 0.3 and 1.1 °C, and annual precipitation approximately 580 mm (Smith et al. 1998).

The area is dominated by coniferous forest with some mixedwood stands in the south. Common tree species include black spruce (*Picea mariana*), jack pine (*Pinus banksiana*), trembling aspen (*Populus tremuloides*), white spruce (*Picea glauca*), balsam fir (*Abies balsamea*), and balsam poplar (*Populus balsamifera*). Poorly drained bogs and fens with moss and shrub layers are also common throughout the area. Deer are found in the southern portion of GHA 26 with some small pockets in the northern half of GHA 26 (Mocker 2015). Boreal woodland caribou (*Rangifer tarandus caribou*) are found within the northern portion of GHA 26,

and throughout GHAs 17 and 17A. Common predators in the study area include wolves and black bears.

Intensity of industrial activity increases from north to south (Figure 2.1). Logging operations previously concentrated in GHA 26 were terminated in September 2009. There was also some historical logging (1984 - 2001) east of the Berens River; otherwise, fire was the main source of disturbance in GHAs 17 and 17A. Licensed moose harvest was suspended in GHA 26 starting in 2010, and rights-based harvest was restricted in approximately 14% of GHA 26 starting in 2012 (Daniel Dupont, Manitoba Conservation and Water Stewardship, personal communication). Licensed bull moose harvest is permitted in GHAs 17 and 17A.

Quantifying disturbance and habitat features

We used ArcGIS 10.2 to create a 4.6 km-radius buffer around each moose sample location based on the median annual home range of five collared moose in eastern Manitoba (Manitoba Conservation and Water Stewardship, unpubl. data, 2012 - 2015). We based buffer size on annual home range instead of summer-fall movements (i.e., when cortisol was integrated into hair) because our samples were collected in winter, and moose winter and summer-fall ranges did not overlap. We clipped buffers at the Manitoba-Ontario border due to limited access to Ontario disturbance data. Within each buffer, we quantified disturbance and habitat features obtained from the Manitoba Land Initiative (http://mli2.gov.mb.ca/) and Manitoba Conservation and Water Stewardship. We determined major road (highways, provincial, municipal, community, and long-term all-weather class 1 and 2 roads), minor road (trails, forestry, mining, park, and short-term all-weather class 3 and 4 roads), and transmission line densities within moose buffers. We also quantified the proportion of logging and fire disturbance between 0 - 10 (recent), 11 - 25 (intermediate), and 26 - 40 (old) years from when individuals were killed or

collared (Courtois et al. 2002; Wasser et al. 2011). When logging and fire areas overlapped, we selected for the most recent of the overlapping regions (e.g., selected a 5 year old logged area over a 20 year old burned area). We also quantified lake, river, and wetland edge within moose buffers because this water habitat provides important submerged vegetation for moose in summer and fall (Courtois et al. 2002; Wasser et al. 2011).

Cortisol analysis

Samples consisted of hide and hair tufts from unspecified body regions. Although cortisol concentrations can vary among body regions (Macbeth et al. 2010), we expected no bias in sample collection. We processed guard hair instead of underfur for cortisol analysis because guard hair would have grown throughout the summer and fall (approximately June – late September; Daniel Dupont, Manitoba Conservation and Water Stewardship, personal communication) when reproduction and mating are demanding periods for moose. Guard hair was also easier to clean and homogenize, and produces less variable cortisol concentrations among and within body regions compared to underfur (Macbeth et al. 2010). Since cortisol becomes integrated into hair as it grows, cortisol concentrations would reflect moose physiology the summer-fall prior to hair collection: summer-fall 2010 to 2014 for samples collected winters 2010-11 to 2014-15.

We washed moose guard hair twice (3 min per wash) using methanol (Appendix A) and dried the hair under a fume hood for at least two days. We then homogenized dried hair into a powder for 0.03 min/mg hair at 30 Hz using a ball mill (Retsch MM 301 Mixer Mill, Retsch Inc, Newtown, Pennsylvania, USA). To extract cortisol from hair, we added 1 mL of methanol per 50 mg hair and placed the samples on a shaker table (Standard Orbital Shaker Model 3500, VWR®) for 24 hours. We centrifuged the samples (15 min at 4500 rpm, at 20°C) and collected the

supernatant. To further extract cortisol, we rinsed the samples with 1 mL of methanol, vortexed them (30 sec), centrifuged them for another 15 minutes (4500 rpm at 20°C), and pooled the supernatants. We repeated the rinsing process two times for a total of three collections per sample (Appendix A). We then dried the pooled supernatant in a concentrator and reconstituted samples with 0.25 - 0.4 mL of radioimmunoassay (RIA) buffer (0.1 M phosphate buffer, 0.9% w/v NaCl, and 0.5% w/v bovine serum albumin).

We used RIA techniques as described by Ryan et al. (2012) to measure cortisol concentrations in hair samples. We combined 100 µL of cortisol-specific antibody (1: 11,000 dilution; Fitzgerald Industries, Acton, MA, USA, product code 20-CR50) with 100 µL of 5000 disintegrations per minute (dpm) of tritiated cortisol tracer (Perkin Elmer, Waltham, MA, USA), and 100 µL of either reconstituted sample or known cortisol standards. Cross-reactivity of the antibody used was 100% for cortisol, 5.7% for 11-deoxycortisol, 3.3% for corticosterone, 36% for prednisolone, and < 0.7% for cortisone. We then let the samples sit at room temperature for one hour and then in a refrigerator overnight (4°C). The next day, we added 100 µL of dextran (0.5% w/v)-coated charcoal (5% w/v), briefly vortexed the samples, and let them incubate for 15 min on ice. We then centrifuged the samples for 30 min (2500 g-force at 4°C) and collected the supernatant into 6 mL scintillation vials. We added 4 mL of Ultima Gold scintillation fluid (Perkin Elmer) and determined radioactivity using a scintillation counter (TriCarb 3100 LSC, Perkin Elmer, Waltham, MA, USA). Using the known cortisol concentrations of a standard curve (13 concentrations ranging from 0.013 to 50 ng/mL), we interpolated cortisol concentrations for each sample. To increase the accuracy of our results, we processed all samples in duplicate and standards in triplicate. Extraction efficiency for pooled wolf hair was 78.75% ± 4.52% (Mean ± SE) based on three recoveries of 0.5 ng cortisol/mL methanol of spiked extract from ground hair.

Cortisol concentrations from serial-diluted pooled moose hair were parallel with serially diluted standard concentrations ($R^2 = 0.99$; $p = 0.003$). Intra-assay coefficient of variability (n = 5) was 6.27% and the inter-assay coefficient of variability (n = 7) was 13.17%.

Data analysis

We knew the age class (calf vs. adult) of a small subset of samples (n = 9) from fall-winter 2014-15 and found no difference in cortisol concentrations between calves and adults while controlling for sex ($F_{1,8} = 1.63$; $p = 0.25$), so we ignored age in further analyses. We created a general linear model to determine the effects of year, sample source, latitude (i.e., north-south gradient in disturbance), and sex on moose cortisol concentrations (log-transformed) to determine the confounding effects of these variables on moose stress response (n = 44). We categorized samples as human-collected (i.e., harvested: n = 19, road-killed: n = 1, or collared: n = 6) or wolf-killed (n = 37) since moose targeted by wolves were potentially more vulnerable (e.g., young, old, diseased, and injured) with higher cortisol concentrations (Appendix B) compared to moose targeted by humans, which were potentially trophy individuals. Within the human-collected category, cortisol concentrations did not vary between harvested and collared moose ($t_{34} = -0.64$; $p = 0.53$). We were unable to include interactions in our model due to unbalanced sample sizes (Table 2.1; Figure 2.2).

We standardized all disturbance and habitat variables to a mean of zero and a standard deviation of one, and determined a priori models (n = 30) based on combinations of disturbance variables thought to affect moose cortisol concentrations, such as recent logging and minor roads. There was no multicollinearity ($|r| < 0.7$) among variables, so we excluded no variables from our models. We used general linear models to examine the effects of disturbance on moose cortisol concentrations (log-transformed; n = 63) while controlling for water edge and sample

source (null model). We controlled for water edge because it provides important forage for moose in summer and fall, and sample source as a potential confounding variable. We then used Akaike's information criterion corrected for small sample sizes (AIC_c) to rank candidate models and calculated the Akaike weight (the relative information content) for each model. We considered models to be good if $\Delta AIC_c < 2$ (Burnham and Anderson 2002) and variables to be informative when zero fell outside the 95% confidence intervals (Arnold 2010). All analyses were completed using JMP® Version 12 (SAS Institute Inc., Cary, North Carolina, USA) using a significance level of $\alpha = 0.05$.

Results

Wolf-killed moose had higher hair cortisol concentrations than human-collected moose ($F_{1,36} = 4.37$, $p = 0.044$; Figure 2.3), but we found no effect of year ($F_{4,36} = 0.80$, $p = 0.536$), latitude ($F_{1,36} = 0.92$, $p = 0.345$), or sex ($F_{1,36} = 0.91$, $p = 0.346$) on moose cortisol concentrations. The overall model, including year, sample source, latitude, and sex, was also significant ($F_{7,36} = 3.69$; $p = 0.0042$).

None of the general linear models containing disturbance variables improved the explanation of log moose hair cortisol concentrations over the null model with just sample source and water edge (Model 1: $R^2 = 0.163$; Table 2.2). Sample source was informative because its 95% confidence interval did not encompass zero ($\beta = -0.422 \pm 0.250$), but water edge was uninformative ($\beta = -0.069 \pm 0.251$). The seven top models were considered good, with $\Delta AIC_c < 2$, but sample source accounted for most of the variation and no disturbance variables were informative.

Discussion

Our results suggest that moose cortisol concentrations were unaffected by disturbance. Although disturbed regions can provide important early successional browse, areas with high human presence and activity, such as highways, can impede moose movements and cause mortality (Laurian et al. 2008; Beyer et al. 2012; Bartzke et al. 2015). Yet human presence and activity in our study area may have been too low to deter moose or cause a stress response. Moreover, habitat types not quantified in this study could have explained some variation in moose cortisol concentrations, particularly regenerating deciduous habitat, which provides important forage for moose (Courtois et al. 2002; Dussault et al. 2005).

Sample source was the greatest predictor of cortisol concentrations, as wolf-killed moose had higher cortisol concentrations than human-collected moose. Although chronic stress is predicted to affect fitness, few studies have demonstrated a direct link between chronic stress and fitness costs in free-ranging wildlife populations. Individuals with high circulating cortisol concentrations might experience muscle wasting, immunosuppression, inhibition of the reproductive axis, growth suppression, and reduced body condition (Boonstra and Singleton 1993; Boonstra et al. 1998; Charbonnel et al. 2008). Charbonnel et al. (2008) found a negative relationship between cortisol concentrations and body condition in vole (*Arvicola scherman*) populations, and Cyr et al. (2007) found that chronically stressed female European starlings (*Sturnus vulgaris*) had lower reproductive success. Results from this study suggest that moose targeted by wolves had higher cortisol concentrations. In particular, we found that moose with high cortisol concentrations were in poorer body condition based on low marrow fat content (Appendix B). Wolves often prey upon individuals that are less fit, including the young, old, diseased and injured (Mech and Peterson 2003; Garrott et al. 2007). Consequently, moose with

reduced body condition, potentially resulting from increased levels of circulating cortisol, could be easier targets for wolves.

Our disturbance variables did not explain significant variation in moose cortisol concentrations, but other factors could have affected moose stress response. In particular, parasites, such as winter tick (*Dermacentor albipictus*; McLaughlin and Addison 1986; Samuel 2007), liver fluke (*Fascioloides magna*; Murray et al. 2006), *Echinococcus granulosus* (Joly and Messier 2004), and meningeal worm (Whitlaw and Lankester 1994) can affect moose health and cause mortality. Indeed, transmission of the meningeal worm from deer to moose has caused moose decline in several regions, including Ontario (Saunders 1973; Whitlaw and Lankester 1994), Nova Scotia (Beazley et al. 2008), and southeastern Manitoba (Lankester 1974). Whitlaw and Lankester (1994) found that moose populations in Ontario decreased when deer densities exceeded 5 deer/ km^2, and where the meningeal worm was abundant in deer feces. Increased deer densities in eastern Manitoba resulting from mild winters could cause greater parasite transmission between deer and moose, thereby increasing moose cortisol concentrations.

Harvest pressure could also impact moose populations in our study region. Licensed moose harvest was cancelled in GHA 26, and rights-based harvest was closed in 14% of GHA 26 in efforts to mitigate moose decline. In other studies, Crichton et al. (2004) found that moose numbers increased from approximately 37 to 142 after road access was denied and hunting was prohibited for seven years in the Happy Lake area of eastern Manitoba. Even with these closures, poaching still occurred (Crichton et al. 2004). Moreover, Rempel et al. (1997) found that moose densities were lower in disturbed regions where roads granted hunters greater access to moose. Although roads at our study site had no direct impact on moose cortisol concentrations, harvest pressure could still affect moose cortisol concentrations, particularly in GHA 26 where humans

are present, and poaching could be a risk. Indeed, we found that wolf cortisol concentrations increased following greater harvest pressure in GHA 26 (Chapter 3).

Predation risk could also cause elevated cortisol concentrations in moose and contribute to population decline (Bergerud et al. 1983; Gasaway et al. 1992). Even with deer present, moose are an important prey source in wolf summer diet in GHA 26 (Mocker 2015). Few deer occur in the northern half of GHA 26, and no deer are in GHAs 17 and 17A, so moose are likely a primary prey source for wolves in these regions. Black bears could also affect moose populations throughout our study region, particularly in spring and summer when calves are born. Indeed, Kotchorek (2002) found a significant increase in moose calf numbers after bears were removed from Hecla Island, Manitoba. Overall, greater wolf and bear predation pressure could cause high cortisol concentrations in moose, particularly where fragmented habitat improves predator hunting efficiency (James and Stuart-Smith 2000) and harsh winters with deep snow increase moose vulnerability to predation (i.e., wolves have lighter foot loading; Gasaway et al. 1983; Nelson and Mech 1986).

Conclusions

Our disturbance variables were not important predictors of moose cortisol concentrations. Factors not examined in this study, such as parasites, predators, and harvest pressure might affect moose cortisol concentrations and should be examined. Quantifying disturbance and specific habitat types (i.e., deciduous forest) within collared moose ranges instead of around single sample locations might also be important in explaining variation in moose cortisol concentrations. Overall, results from this study provide an important link between chronic stress and health costs in a free-ranging mammal. Determining the chronic stress response of wildlife

can be useful for assessing individual health and condition, particularly with implementation of recovery strategies, changes in disturbance regimes, and climate warming.

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Table 2.1. Distribution of moose (*Alces alces*) samples by year, sex, and source (wolf-killed vs. human-collected) in eastern Manitoba.

	Wolf		**Human**	
	Male	**Female**	**Male**	**Female**
2010	0	1	0	0
2011	0	0	7	2
2012	0	0	0	5
2013	0	8	2	0
2014	2	7	10	0

Table 2.2. Comparison of general linear models* with disturbances explaining moose (*Alces alces*) cortisol concentrations (log-transformed) based on Akaike's information criterion corrected for small sample sizes (AICc). The top 20 of 30 models are presented below, including the null and global models. All models control for sample source and water edge.

Model	Model variables	R^2	SSE	*N*	*K*	AICc	ΔAICc	Relative L	AICc Weight
1	Null model (sample source and water edge)	0.163	51.88	63	4	-3.55	0.00	1.00	0.12
2	Fire $_{intermediate}$	0.186	50.45	63	5	-2.94	0.60	0.74	0.09
3	Roads $_{minor}$	0.181	50.75	63	5	-2.57	0.98	0.61	0.07
4	Logging $_{intermediate}$	0.174	51.20	63	5	-2.01	1.53	0.46	0.06
5	Logging $_{recent}$	0.174	51.21	63	5	-2.00	1.54	0.46	0.05
6	Fire $_{recent}$	0.173	51.29	63	5	-1.90	1.64	0.44	0.05
7	Transmission lines	0.171	51.40	63	5	-1.77	1.78	0.41	0.05
8	Fire $_{recent}$, fire $_{intermediate}$	0.199	49.67	63	6	-1.48	2.07	0.36	0.04
9	Roads $_{major}$	0.165	51.76	63	5	-1.33	2.22	0.33	0.04
10	Fire $_{old}$	0.164	51.83	63	5	-1.24	2.30	0.32	0.04
11	Logging $_{old}$	0.163	51.88	63	5	-1.18	2.36	0.31	0.04
12	Logging $_{recent}$, roads $_{minor}$	0.194	49.95	63	6	-1.12	2.42	0.30	0.04
13	Fire $_{intermediate}$, logging $_{intermediate}$	0.194	49.97	63	6	-1.10	2.45	0.29	0.03
14	Fire $_{intermediate}$, fire $_{old}$	0.191	50.17	63	6	-0.85	2.70	0.26	0.03
15	Logging $_{old}$, roads $_{minor}$	0.188	50.35	63	6	-0.62	2.92	0.23	0.03
16	Fire $_{recent}$, logging $_{recent}$	0.187	50.39	63	6	-0.57	2.97	0.23	0.03
17	Roads $_{minor}$, transmission lines	0.185	50.52	63	6	-0.41	3.14	0.21	0.02
18	Logging $_{recent}$, logging $_{intermediate}$	0.185	50.54	63	6	-0.38	3.16	0.21	0.02
19	Logging $_{intermediate}$, roads $_{minor}$	0.182	50.74	63	6	-0.13	3.41	0.18	0.02
20	Logging $_{intermediate}$, logging $_{old}$	0.177	51.04	63	6	0.24	3.78	0.15	0.02
30	Global	0.263	45.69	63	13	13.19	16.73	0.00	0.00

*****Models are summarized by the sum of square error (SSE), sample size (*N*), number of parameters (*K*), AICc score, the difference between the AICc for that model and the lowest AICc for any model (ΔAICc), the relative likelihood (Relative L), and AIC_c weight (model probability).

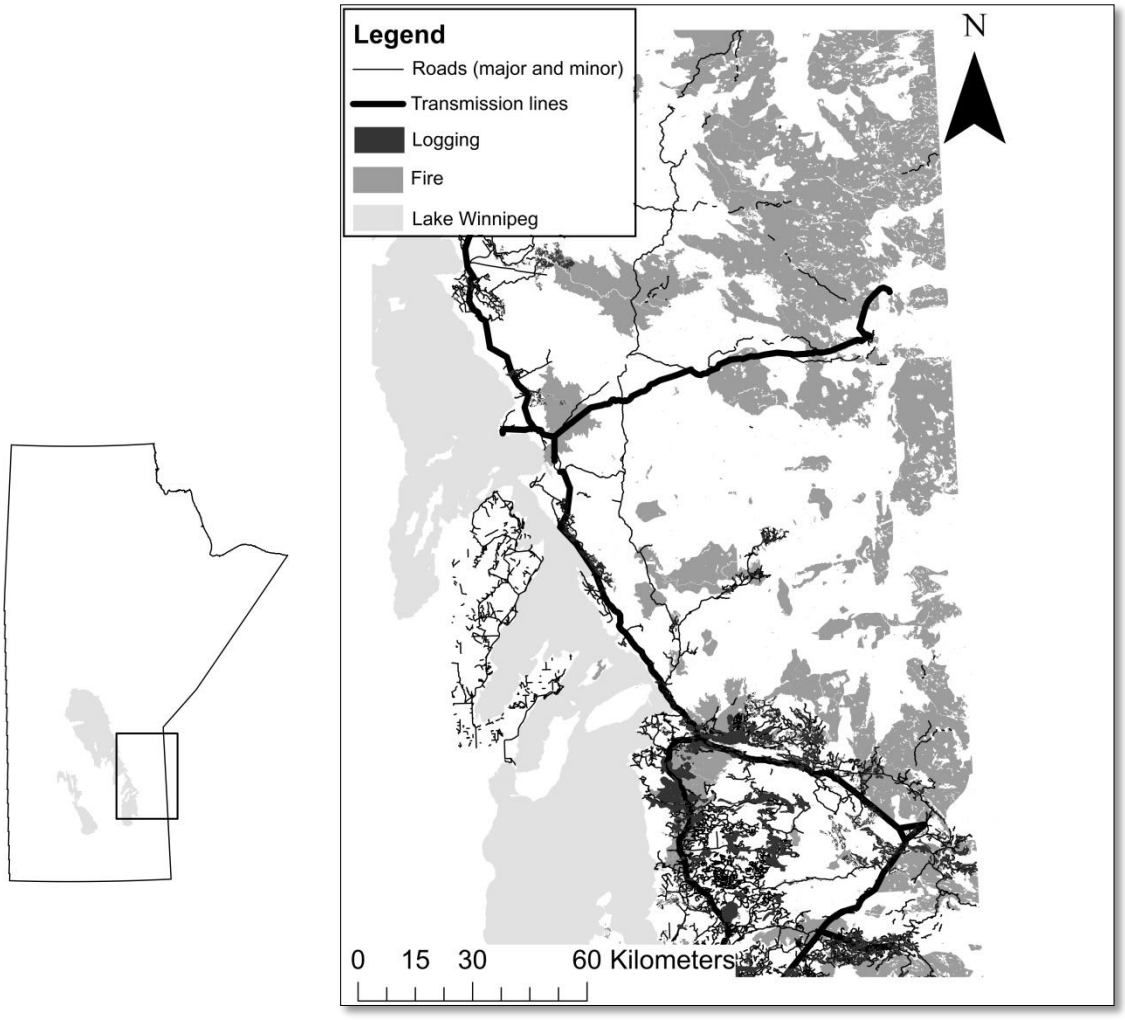

Figure 2.1. Distribution of disturbances within moose (*Alces acles*) study region in eastern Manitoba, including roads, transmission lines, and logging (1971 - 2009) and fire (1971 - 2014).

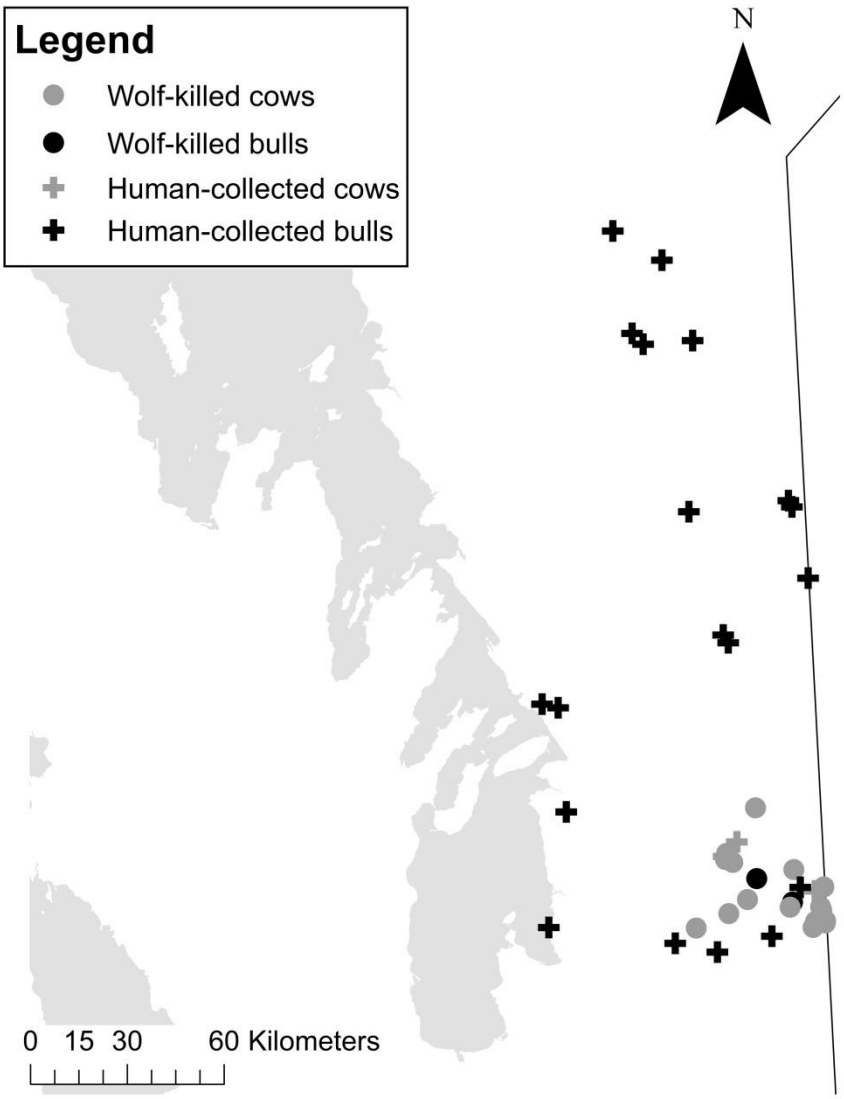

Figure 2.2. Distribution of moose (*Alces alces*) samples by latitude, sex, and source in eastern Manitoba.

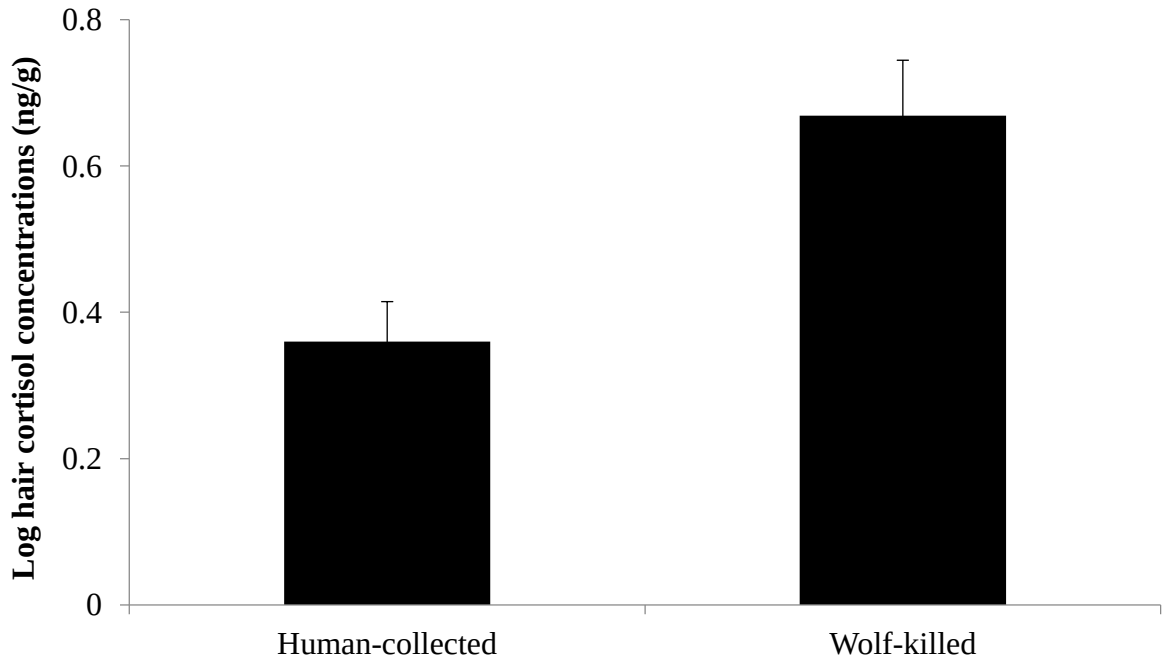

Figure 2.3. Cortisol concentrations (log-transformed, mean ± SE) of human-collected (n = 37) and wolf-killed (n = 26) moose (*Alces alces*) in eastern Manitoba.

Chapter 3: Stress response of wolves to harvest pressure, winter severity, and diet in eastern Manitoba

Abstract

Chronic (long-term) stress can be harmful to the survival and reproduction of social carnivores, particularly if their complex social relationships are disturbed. Winter severity can also affect a predator's ability to attain prey, and injuries acquired when hunting aggressive prey can affect social status of carnivores, all of which could increase chronic stress. We assessed the chronic stress response of wolves (*Canis lupus*) to increased harvest pressure, winter severity, and diet. We measured cortisol concentrations in the guard hair of 47 hunted, trapped, or road-killed wolves between 2011 and 2013 in eastern Manitoba. We also estimated the proportion of moose and deer in wolf diet using stable isotope analysis and the Bayesian mixing model MixSIAR. Wolf cortisol concentrations were higher in 2012 and 2013 compared to 2011 following increased harvest pressure, but this annual variation was not explained by winter severity. Females had higher cortisol concentrations than males, but the proportion of moose and deer in wolf diet did not affect wolf cortisol concentrations. Based on our results, determining the chronic stress of wolves can be useful for assessing population health, particularly with increased harvest pressure.

Introduction

Carnivore abundance, behaviour, and condition can be affected by a number of factors, including human activity (Boydston et al. 2003; Rich et al. 2012), harvest pressure (Bryan et al. 2015; Molnar et al. 2015), climate (Fuller 1991; Trinkel 2013), intra-specific competition (Sands and Creel 2004; Rich et al. 2012), and changes in prey availability and abundance (Honer et al. 2005; Bissett et al. 2012; Bryan et al. 2013). Unlike solitary carnivores, social carnivores are also sensitive to disturbance and other factors that affect their social status, relationships, and group

dynamics (Macdonald 1983; Brainerd et al. 2008; Borg et al. 2015; Molnar et al. 2015). While studies have examined the effects of disturbance on abundance and behaviour of social carnivores (Mills and Knowlton 1991; Honer et al. 2005; Bryan et al. 2015), few studies have looked at the mechanistic impact. Since chronic (long-term) stress can affect an animal's ability to survive, reproduce, and respond to disease (Boonstra and Singleton 1993; Cyr et al. 2007; Charbonnel et al. 2008), determining the chronic stress response of social carnivores to factors that affect their complex social structure and relationships is important for assessing population health.

In mammals, the stress response is controlled by the hypothalamic-pituitary-adrenal axis, which releases glucocorticosteroids, such as cortisol, into the blood (Boonstra and Singleton 1993; Boonstra 2004; Reeder and Kramer 2005). Circulating glucocorticosteroids elicit the mobilization of glucose, which allows an animal to respond quickly to a stressor in its environment and return to a homeostatic state (Boonstra and Singleton 1993; Boonstra 2004; Reeder and Kramer 2005). Although short-term increases in the circulation of glucocorticosteroids can be adaptive for an organism, long-term increases of circulating glucocorticosteroids resulting from chronic disturbance in the environment can be detrimental to an organism's ability to survive and reproduce (Boonstra and Singleton 1993; Boonstra et al. 1998; Cyr et al. 2007; Charbonnel et al. 2008). Studies have focused on measuring glucocorticosteroids in plasma, saliva, urine, and feces; however, these tissues provide only short-term measures of stress that can be confounded by time of day, diet, and manipulation of the animal (Davenport et al. 2006; Macbeth et al. 2010; Ashley et al. 2011). Recently studies have validated and used hair to measure steroid concentrations in free-ranging mammals (Macbeth et al. 2010; Bechshoft et al. 2013; Bryan et al. 2015). Hormones such as cortisol

become integrated into the hair as it grows, thereby providing an indication of chronic stress (Macbeth et al. 2010).

The stress response of wolves (*Canis lupus*) to factors such as harvest pressure, changes in prey availability and abundance, climate variability, and intra-specific competition is not well-understood. Wolves are highly social and typically live in packs with one breeding pair and their offspring from the previous 1 - 3 years (Mech 1999). The pack acts as a unit year-round with the breeders leading pack activities, including food gathering and caring for young (Mech 1999). Disruption of a pack's complex social structure could cause a stress response in individuals because it affects their reproductive, hunting, and territorial behaviours, ability to learn, and genetic structure (Haber 1996; Borg et al. 2015; Bryan et al. 2015; Molnar et al. 2015). Indeed, Bryan et al. (2015) found that cortisol concentrations in hair were higher for a wolf population that experienced greater harvest pressure, and suggested that removing individuals from the population affected their social structure. Molnar et al. (2015) also found that fecal cortisol concentrations were higher in wolves that lived in socially disrupted packs. Likewise, Eggermann et al. (2013) found that social interactions during breeding had a greater impact on wolf fecal cortisol concentrations than human traffic, pack size, and prey density.

Weather is also an important factor affecting wolf-prey dynamics (Mech and Peterson 2003). Indeed, the most important condition affecting wolves and their prey is snow (Mech and Peterson 2003). Although wolf cortisol hormones are integrated into hair in summer-fall as hair grows, atypical snow and climate conditions in the prior winter might affect the wolf's stress response into summer-fall (i.e., potential for carry-over effects). In particular, deep and dense snow can hinder movements of ungulates and wolves (Kelsall 1969; Nelson and Mech 1986); however, wolves have lighter foot-loading compared to ungulates, and so they sink less in deep

snow (Kelsall 1969; Nelson and Mech 1986). Snow resistance, including deep and hard snow, can also affect an ungulate's ability to forage on vegetation (Mech and Peterson 2003). If an ungulate has reduced access to vegetation, its body condition could decrease, making it vulnerable to wolf predation (Mech and Peterson 2003). Thus, wolves could benefit from severe winters if ungulate vulnerability increases (Sands and Creel 2004). Alternatively, when winter conditions are mild (i.e., shallow snow) wolves might travel farther to find food, spend less time interacting with other pack members, kill less, and scavenge more (Nelson and Mech 1986; Fuller 1991; Huggard 1993). An increased stress response during a mild winter could carry over into summer if the energetic demand associated with finding food outweighs the energetic intake of food (McEwan and Wingfield 2002; Sanderson et al. 2014).

The anti-predator defences of prey can also affect a wolf's stress response. Wolves are opportunistic generalists and will eat a range of foods, including ungulates, small mammals, fish, carrion, and even garbage (Fuller and Keith 1980; Peterson and Ciucci 2003). Wolves can show preference for certain prey, depending on the abundance of that prey and their body size, age, sex, anti-predator defenses, and vulnerability (Fuller and Keith 1980; Potvin et al. 1988; Mech and Peterson 2003; Garrott et al. 2007). Wolves often take down individuals that are less fit, including the young, old, diseased or injured (Fuller and Keith 1980; Potvin et al. 1988; Mech and Peterson 2003). While tradition and learning play some role in prey selection, a combination of capture efficiency and nutritional value of prey relative to risk are the most important factors affecting wolf prey choice (Mech and Peterson 2003). Most prey are adapted to escape wolf predation in some way, with some species injuring or even killing wolves during attack. White-tailed deer (*Odocoileus virginianus*), pronghorn (*Antilocapra americana*), sheep (*Ovis aries*), and hares (*Lepus americanus*) predominantly rely on flight to escape wolves, inflicting little injury

during attack (Mech and Peterson 2003), whereas moose (*Alces alces*), bison (*Bison bison*), and muskoxen (*Ovibos moschatus*) are large and aggressive (Carbyn and Trottier 1987; Mech and Peterson 2003; Garrott et al. 2007), and will attack with their hooves, horns, and/or antlers, potentially injuring or killing a wolf. Cow moose, in particular, will attack wolves violently to protect their newborn calves, sometimes resulting in battles that can last for days (Mech and Peterson 2003). Injuries acquired by wolves can affect their social status (Garrott et al. 2007), and so the risk of injury or death when attacking aggressive prey, such as moose, could increase a wolf's stress response.

Our objective was to determine the effects of harvest pressure, winter severity, and diet on wolf cortisol concentrations in eastern Manitoba. We predicted greater cortisol concentrations in wolves following greater harvest pressure and milder winters. We also predicted that wolves with a greater proportion of moose (aggressive) in their diet would have higher cortisol concentrations than wolves eating mainly deer (non-aggressive).

Methods

Study area

Wolf hair samples were collected in southeastern Manitoba (Game hunting area (GHA) 26; centroid: 50° 40' 48" N and 95° 45' 14" W; Figure 3.1). The region's climate is marked by short, warm summers and long, cold winters with mean annual temperatures ranging from 0.3 to 1.1 °C (Smith et al. 1998). Mean annual precipitation is about 580 mm, but can vary a lot among years (Smith et al. 1998). The study region is found within the boreal shield and is dominated by coniferous forest intermixed with peatlands, bogs, and fens (Smith et al. 1998). Black spruce (*Picea mariana*) is the dominant tree species, but jack pine (*Pinus banksiana*) is found commonly on upland sites (Smith et al. 1998).

Moose and boreal woodland caribou are found primarily in the northern half of GHA 26, whereas deer are concentrated in the south with some small pockets in the north (Mocker 2015). Moose and deer tend to occupy early successional habitat or disturbed (fire or anthropogenic) areas (Monthey 1984; Fisher and Wilkinson 2005), whereas boreal woodland caribou occupy old successional and treed muskeg habitat to separate themselves from moose, deer, and wolves (Bradshaw et al. 1995; Stuart-Smith et al. 1997). Smaller prey, including snowshoe hare and beaver, are also distributed throughout the study region.

Sample collection

Wolf hair samples (n = 47) were collected by Manitoba Conservation and Water Stewardship from registered trappers, hunters, and road kill between October and March (winter) from 2011-12 to 2013-14. Wolf harvest pressure increased following the enactment of the wolf incentive program in winter 2011-12 in GHA 26. The program was developed to help recover declining moose populations by increasing the bag limit to two wolves per licensed hunter, and providing trappers with a $250 incentive per wolf harvested from a registered trap line.

Samples consisted of hide and hair tufts from unspecified body regions. Hair growth can vary between body regions, potentially affecting hormone deposition (Macbeth et al. 2010; Bryan et al. 2015). We attempted to compare cortisol concentrations across body regions of wolf carcasses, but our sample size was too small (n = 2; Appendix A). Nonetheless, we expected no bias in any one body region among years. Ages of wolves were also unknown, but we also expected no age bias among years. Indeed, Molnar et al. (2015) found that age had no effect on fecal cortisol concentrations in wolves. Finally, pigmentation can play a role in incorporating steroids into hair (Pragst and Balikova 2006). We examined whether cortisol concentrations (log-

transformed) varied among hair colour (white, medium (brown or grey), and black), and found no difference ($F_{2, 60} = 2.38$; $p = 0.102$; $n = 63$).

We processed wolf guard hair for cortisol and stable isotope analyses because guard hair grows throughout mid-summer and late-fall (Darimont et al. 2003; Urton and Hobson 2005) when moose consumption is greater (i.e., moose calves are vulnerable; Mocker 2015). Guard hair was also easier to clean and homogenize than underfur (Macbeth et al. 2010).Cortisol and diet information become integrated into guard hair as it grows, so samples collected in winter would reflect wolf physiology and diet the summer-fall prior to hair collection: summer-fall 2011, 2012, and 2013.

To reconstruct wolf diet, we also collected tissue samples from common prey in southeastern Manitoba (Mocker 2015). Moose, white-tailed deer, beaver, and snowshoe hare muscle samples were collected by Manitoba Conservation and Water Stewardship from registered trappers, hunters, and road kill in the study area. Boreal woodland caribou hair was collected from collared individuals in the study area.

Winter severity index

To assess if winter severity affected wolf cortisol concentrations, we used a winter severity index (WSI) that reflected the extent to which winter conditions affected the overwinter survival of white-tailed deer. This index adds a point for each day temperatures were cooler than – 7°C and an additional point for each day snow depths were ≥ 35 cm (Brinkman et al. 2005). Winter indices < 100 indicate a mild winter, 100 - 180 indicate a moderate winter, and > 180 indicate a severe winter. Although the index was calculated for deer, we expected that severe winters would have similar effects on other ungulates, such moose.

Stable isotope analysis

Stable isotope ratios of a consumer reflect the stable isotope ratios of its prey (Peterson and Fry 1987; Derbridge et al. 2012). Unlike scat and stomach content analyses, stable isotope ratios provide a direct measure of nutrients assimilated into a consumer's tissues (Peterson and Fry 1987). Carbon and nitrogen stable isotope ratios vary between food sources, so they can be used to reconstruct wolf diet. Measures of $\delta^{13}C$ vary among marine, freshwater, and terrestrial prey, as well as between plants with C_3 or C_4 photosynthetic pathways (DeNiro and Epstein 1978; Ben-David et al. 2001), whereas measures of $\delta^{15}N$ vary among trophic positions (DeNiro and Epstein 1981; Peterson and Fry 1987).

We washed sub-sampled wolf guard hair with soapy water (Appendix A), thoroughly rinsed the hair with a high-pressure wash, dried the hair at 60°C, and homogenized it using scissors or a ball mill (30 Hz; Retsch MM 301 Mixer Mill, Retsch Inc, Newtown, Pennsylvania, USA). We freeze-dried prey muscle for 48 hours and then homogenized samples using a mortar and pestle. Since lipids can influence stable carbon isotope ratios, we removed lipids from ground muscle using a Soxhlet apparatus with petroleum ether for a minimum of eight hours. We then weighed powdered hair (0.6 to 0.8 mg) or muscle (0.4 to 0.6 mg) into 6 x 4 mm tin capsules and shipped the samples to the Chemical Tracers Laboratory, Great Lakes Institute for Environmental Research, University of Windsor for measurement of stable isotope ratios on a continuous flow isotope ratio mass spectrometer. During this process, samples were combusted and separated into CO_2 and N_2, which were measured to determine carbon and nitrogen stable isotope ratios (Derbridge et al. 2012). These values were then presented in delta notation by comparing the stable isotope ratios to an international standard, PeeDee Belemnite limestone for carbon and atmospheric N_2 for nitrogen (DeNiro and Epstein 1978, 1981).

Cortisol analysis

We removed contaminants from hair using two 3-min methanol washes (Appendix A) and dried the hair under a fume hood for at least two days. We then ground the hair into a powder for 0.03 min/mg hair at 30 Hz using a ball mill, added 1 mL of methanol per 50 mg hair, and placed the samples on a shaker table for 24 hours (Standard Orbital Shaker Model 3500, VWR®). After extracting cortisol, we centrifuged the samples (15 min at 4500 rpm, at 20°C) and collected the supernatant. We then rinsed the samples with 1 mL of methanol, vortexed them (30 sec), centrifuged them for another 15 minutes (4500 rpm at 20°C), and pooled the supernatants (Appendix A). We repeated this process two times for a total of three collections per sample. We then dried the pooled supernatant in a sample concentrator and reconstituted samples with 0.4 - 0.6 mL of radioimmunoassay (RIA) buffer on the day of the assay (0.1 M phosphate buffer, 0.9% w/v NaCl, and 0.5% w/v bovine serum albumin).

We used RIA techniques as described by Ryan et al. (2012) to measure cortisol concentrations in hair samples. We combined 100 µL of cortisol-specific antibody (1:9000 dilution; Fitzgerald Industries, Acton, MA, USA, product code 20-CR50) with 100 µL of 5000 disintegrations per minute (dpm) of tritiated cortisol tracer (Perkin Elmer, Waltham, MA USA), and 100 µL of either reconstituted sample or known cortisol concentrations (cortisol standards). Cross-reactivity of the antibody used was 100% for cortisol, 5.7% for 11-deoxycortisol, 3.3% for corticosterone, 36% for prednisolone, and < 0.7% for cortisone. We let the samples incubate for one hour at room temperature and then overnight at 4°C. The following day, we added 100 µL of dextran (0.5% w/v)-coated charcoal (5% w/v), briefly vortexed the samples, and let them incubate for 15 min on ice. We then centrifuged the samples for 30 min (2500 g-force at 4°C), collected the supernatant, added 4 mL of Ultima Gold scintillation fluid (Perkin Elmer), and determined radioactivity using a scintillation counter (TriCarb 3100 LSC, Perkin Elmer,

Waltham, MA, USA). Using the known cortisol concentrations of a standard curve (13 concentrations ranging from 0.013 to 50 ng/mL), we interpolated cortisol concentrations for each sample. To increase the accuracy of our results, we processed all samples in duplicate and standards in triplicate. Extraction efficiency for pooled wolf hair was 88.88% ± 6.00% (Mean ± SE) based on six recoveries of 0.2 ng cortisol/mL methanol of spiked extract from ground hair. Cortisol concentrations from serial-diluted pooled wolf hair were parallel with serially diluted standard concentrations ($R^2 = 0.93$; $p = 0.036$). Intra-assay coefficient of variability (n = 10) was 9.66%, and inter-assay coefficient of variability (n = 6) was 15.32%.

Data analysis

We used a hierarchical Bayesian stable isotope mixing model, MixSIAR (Stock and Semmens 2013), to estimate the proportion of prey in wolf diet between summer-fall 2011 and 2013. This program estimates the proportional diet of a consumer while accounting for variability in prey stable isotope ratios and trophic discrimination (Moore and Semmens 2008; Derbridge et al. 2012). We used $\delta^{13}C$ and $\delta^{15}N$ values of wolf guard hair and prey muscle (deer, moose, beaver, and hare) in our models, since wolves assimilate nutrients from prey muscle. We did not have samples of caribou muscle, so we used caribou hair and applied a correction factor for nitrogen differences between hair and muscle (+0.3; Sponheimer et al. 2003a, b; Mocker 2015). Carbon and nitrogen isotope signatures of prey differed, so we were able to include each prey as a distinct source in our diet model (Mocker 2015). We also found no annual differences in isotopic signatures of prey, so we combined samples from various years (Mocker 2015).

To determine diet using stable isotope analysis, a trophic discrimination (difference in stable isotope ratios between consumer and prey) correction must be applied to prey isotope signatures prior to comparison with consumer signatures. Mocker (2015) conducted a sensitivity

analysis comparing three sets of trophic discrimination factors from controlled feeding studies, and found that the red fox hair discrimination factor (Roth and Hobson 2000) corrected for body size provided the best representation of wolf and prey tissue discrimination ($\Delta C = 2.6 \pm 0.1$, $\Delta N = 2.9 \pm 0.1$).

In our model, we included wolf ID as a categorical fixed effect to determine individual wolf diet estimates, specified process only error (necessary with only one data point per fixed effect), and selected very long chains (i.e., three chains with 1,000,000 iterations/chain, with a burn-in of 500,000, and thinned every 500^{th} iteration). We did not include informative priors (prior distributions produced from outside knowledge, such as research, natural observations, and professional opinions) in our diet model since these values could have a strong influence on individual wolf diet estimates. The model produced a distribution of diet estimates for each wolf (the proportion of diet from each prey species), and we used the median of each distribution to represent the contribution of that prey species to that wolf's diet.

We log-transformed wolf cortisol concentrations for normalization and used a general linear model to determine the effects of harvest, winter severity, and diet on wolf stress response. We were unable to examine the direct effects of harvest and winter severity on wolf cortisol concentrations due to our small annual sample size (n = 3 years). To test the prediction that wolf cortisol concentrations increased following increased harvest pressure, we compared cortisol concentrations the summer prior to the start of the incentive program (winter 2011-12) with the following two years. We also accounted for sex and tested the interaction between year and sex. We examined how wolf cortisol concentrations varied annually with deer WSI values to test the prediction that wolves have higher cortisol concentrations when winters are mild. We also examined how consumption of moose (aggressive) vs. deer (non-aggressive) affected wolf

cortisol concentrations. We conducted all analyses using JMP® Version 12 (SAS Institute Inc., Cary, North Carolina, USA) using a significance level of $\alpha = 0.05$.

Results

Our model with year, sex, and median proportion of moose and deer in wolf diet had a significant effect on wolf hair cortisol concentrations ($F_{7, 39} = 4.44$; $p = 0.001$). Wolf hair cortisol concentrations varied annually ($F_{2, 39} = 10.96$; $p = 0.0002$; Figure 3.2), with lower concentrations in 2011 compared to 2012 (Tukey HSD, $p = 0.022$) and 2013 ($p = 0.0002$; concentrations did not differ between 2012 and 2013, $p = 0.524$). Following enactment of the wolf incentive program, wolf harvest increased threefold in 2011-12 and almost twofold in 2012-13 compared to 2010-11 (Table 3.1). Winters 2010-11 and 2012-13 were severe (WSI > 180) and winter 2011-12 was mild (WSI < 100; Table 3.1), so winter severity was unrelated to annual variation in wolf hair cortisol concentrations. Females had higher cortisol concentrations than males ($F_{1, 39} = 4.18$; $p = 0.048$), with no interaction between year and sex ($F_{2, 39} = 1.64$; $p = 0.207$). Mean ± SD stable isotope ratios of wolf hair were $\delta^{13}C = -23.29 \pm 0.60$ and $\delta^{15}N = 5.96 \pm 0.84$, and median % moose in wolf diet averaged 17 ± 9%. Other than moose, deer were the most common prey (24 ± 11%). Wolf hair cortisol concentrations were unaffected by the proportion of moose ($F_{1, 39} = 0.91$; $p = 0.346$) and deer ($F_{1, 39} = 1.02$; $p = 0.320$) in their diet (Figure 3.3).

Discussion

Cortisol concentrations were high following the enactment of the wolf incentive program in winter 2011-12, supporting the wolf harvest hypothesis. Similarly, Bryan et al. (2015) found that cortisol concentrations in hair were higher in wolves that experienced greater hunting pressure, and suggested that hunting disrupted their complex social structure. Moreover, Molnar et al. (2015) found higher cortisol concentrations in wolf feces from socially disrupted packs. In

another study, Gobush et al. (2008) found that poaching disrupted social status and interactions among female elephants (*Loxodonta africana*), causing an increased stress response. Group living can be beneficial for a number reasons, including cooperative hunting (Sand et al. 2006; MacNaulty et al. 2011), improved foraging efficiency (Kie 1999), and defending territories (Mosser and Packer 2009). Thus, disruption of groups through harvest can affect social dynamics, resulting in elevated cortisol concentrations in individuals. In particular, removing wolves from a population can alter predation patterns, increase reproductive rates, and increase time spent defending kill sites (Borg et al. 2015; Bryan et al. 2015). Removal of individuals can also disrupt the transfer of learning among generations, resulting in fewer and simpler learned behaviours (Haber 1996). Wolves typically live 7 - 10 years in structured packs, but with long-term harvest pressure, few wolves live beyond 5 - 7 years (Hayes et al. 1991; Haber 1996). Thus, long-term harvest pressure can result in high population turnover and young age structure, affecting the transfer of knowledge among generations. Alternatively, greater harvest pressure could result in more solitary wolves in the population that have higher cortisol concentrations due to lack of social interactions.

Annual differences in cortisol concentrations were not explained by winter severity. Similarly, Sands and Creel (2004) found that variation in snow pack (mass of snow) and temperature did not affect wolf fecal cortisol concentrations. Wolves can hunt in small groups or alone, increasing their encounter rates with prey during mild winters (Fuller 1991). Wolves are also opportunistic generalists and diet switching allows them to adjust to a changing environment if one of their primary food sources becomes unavailable or difficult to catch. In eastern Manitoba, wolves predominantly consume deer and moose in winter (Mocker 2015), but other available prey include boreal woodland caribou, snowshoe hare, beaver, and other small

mammals. The higher nitrogen signatures in some wolves could have resulted from consumption of livestock, fish by-catch, or human garbage (DeNiro and Epstein 1981; Mocker 2015). Although livestock predation is not commonly reported in the area, fisherman and local residents have witnessed wolves scavenging fish by-catch off Lake Winnipeg, as well as fish remains and garbage in dumps (Mocker 2015). Moreover, trappers often use fish remains to bait traps, which could have also provided food for wolves in winter. Consequently, wolf cortisol concentrations might have been less affected by ungulate catchability during mild winters if they were able to hunt other prey, or scavenge on carrion and garbage to maintain good body condition.

Diet also had no effect on wolf cortisol concentrations, suggesting that prey type and anti-predator defenses do not affect wolf stress response in eastern Manitoba. Greater pack size might alleviate stress by dividing hunting efforts among multiple individuals (Hayes et al. 2000). Large wolf packs (e.g., four wolves) are more likely to attack bigger and more aggressive prey compared to small packs (e.g., two wolves) or solitary wolves (Hayes et al. 2000). Thus, an individual's risk of injury might be lower if more wolves cooperatively hunt a moose. However, large packs are not necessary to take down moose considering some solitary wolves and small wolf packs hunt moose regularly (Thurber and Peterson 1993). Wolves are quick to learn the behaviours of their prey, and develop habits and traditions that maximize predation efficiency (Mech and Peterson 2003). Wolves can also assess the vulnerability of individuals and select for young, old, injured, or diseased animals (Chapter 2). Mocker (2015) suggested that wolves might consume more moose calves in summer, or naive yearlings that became independent from their cows. Thus, wolves that hunt moose might not experience higher cortisol concentrations if they hunt in large packs, are experienced hunters, or target vulnerable individuals. Furthermore, the

benefit of attaining more meat when attacking moose compared to smaller prey, such as deer, hare, and beaver, might outweigh the threat of injury during attack.

Wolf cortisol concentrations were also higher in females compared to males. Bryan et al. (2015) found that female wolves tended to have higher cortisol levels than males; however; Sands and Creel (2004) and Molnar et al. (2015) found no difference in fecal cortisol concentrations between sexes of wolves. Considering one male and female wolf typically reproduce in a pack, and subordinate males and females have similar roles (i.e., caring for young and gathering food; Mech 1999), we did not expect differences in cortisol concentrations between sexes. An explanation for these differences could be that female canids have higher baseline cortisol concentrations than males (Reeder and Kramer 2005; McDonald 2013). Indeed, Reeder and Kramer (2005) suggest that high baseline hormone concentrations are a common trend in female mammals.

Conclusions

Increased harvest pressure was the most plausible explanation for annual variation in wolf cortisol concentrations in eastern Manitoba, although we only had samples for one year prior to the enactment of the incentive program. Bryan et al. (2015) and Molnar et al. (2015) also acknowledged that factors, such as changes in prey availability and abundance, climate, and differences between habitats and sampling procedures, could affect wolf cortisol concentrations. We measured cortisol concentrations from the same habitat using the same sampling methods for all three years, and winter severity had no effect on wolf cortisol concentrations. We did not estimate prey abundance and availability directly, but we expected that any major changes in prey abundance or availability would have been reflected in wolf diet, particularly if its preferred prey became unavailable. Eggermann et al. (2013) found that prey availability was not a good

predictor of wolf cortisol concentrations, and Molnar et al. (2015) found no variation in cortisol concentrations among packs consuming different prey within the same study region. We found that wolves consumed mainly deer between summers 2011 and 2013 with no obvious prey switching in their diet. Mocker (2015) found that wolves mainly consumed moose and beaver from summers 2011 to 2013 in our study region; however, she examined group diet instead of individual wolf diets and included informative priors in her mixing model.

The stress response of wildlife has been recognized as an important indicator of health and condition (Wikelski and Cooke 2006; Charbonnel et al. 2008). Although wolf populations are currently stable in Manitoba, monitoring cortisol concentrations will be important with increasing harvest pressure. Indeed, measuring cortisol concentrations in hair can provide a simple and non-invasive technique for monitoring the physiological effects of disturbance on free-ranging mammals.

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Table 3.1. Annual wolf (*Canis lupus*) harvest and winter severity indices from winters 2010-11 to 2012-13 in eastern Manitoba.

Winter	Wolves harvested	Winter severity index
2010-11	19	199
2011-12	57	90
2012-13	34	199

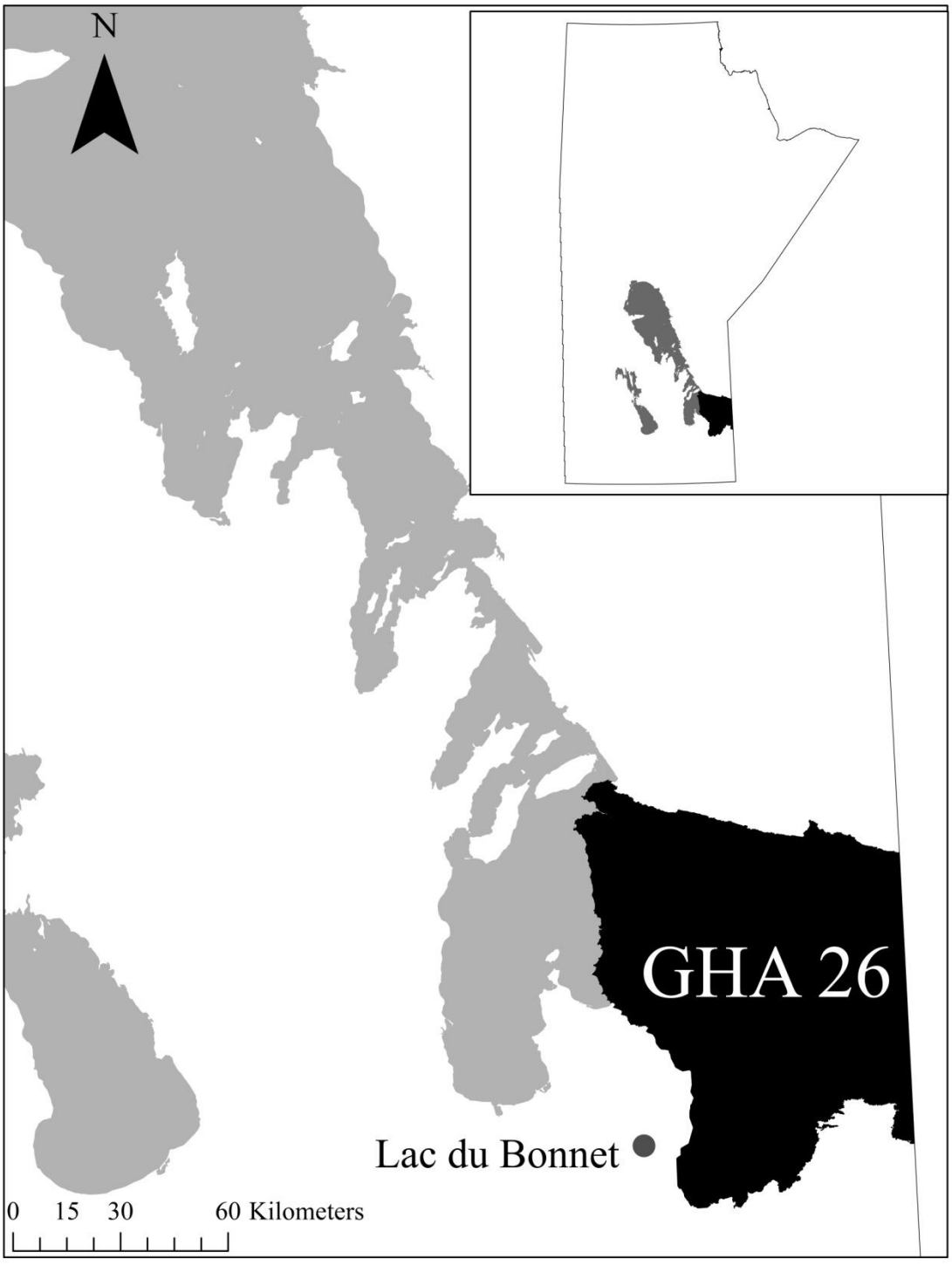

Figure 3.1. Game hunting area (GHA) 26 where wolf (*Canis lupus*) hair samples were collected in eastern Manitoba.

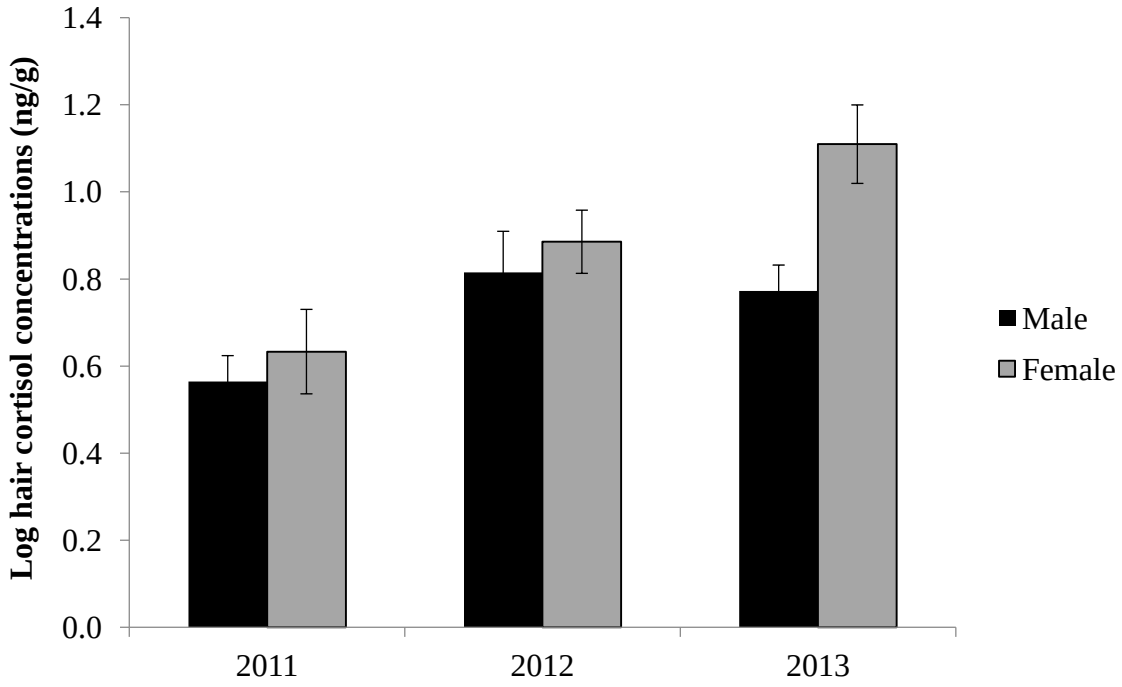

Figure 3.2. Cortisol concentrations (log-transformed, mean ± SE) of male (2011: n = 12; 2012: n = 5; 2013: n = 8) and female (2011: n = 10; 2012: n = 5; 2013: n = 7) wolves (*Canis lupus*) in eastern Manitoba.

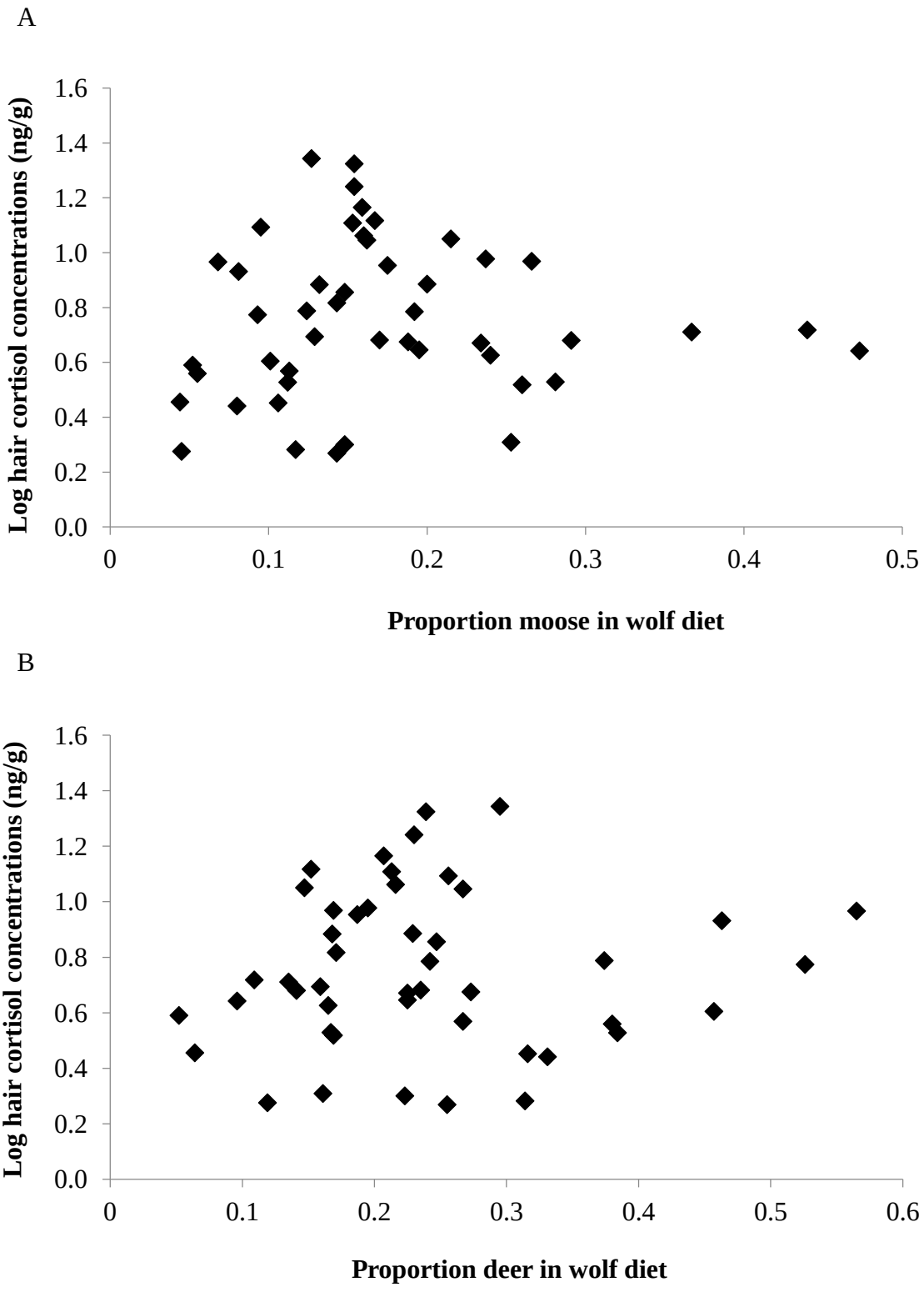

Figure 3.3. Correlation between wolf (*Canis lupus*) cortisol concentrations (log-transformed) and median proportion of (A) moose and (B) deer in wolf diet from summer-fall 2011 to 2013 in eastern Manitoba.

Thesis conclusion

We investigated the effects of disturbance on the stress response of boreal woodland caribou (*Rangifer tarandus caribou*; hereinafter referred to as caribou), moose (*Alces alces*), and wolves (*Canis lupus*) in eastern Manitoba. Caribou are considered sensitive to disturbance that deters their movements and increases their interactions with other ungulates, predators, and humans (Bergerud and Elliot 1986; Seip 1992). Consistent with that notion, we found that intermediate (6 – 21 years) logging caused elevated cortisol concentrations in caribou. Alternatively, moose can benefit from minor disturbance that increases early successional browse, although major disturbance (e.g., highways) can deter moose movements and cause mortality (Laurian et al. 2008; Beyer et al. 2012; Bartzke et al. 2015). However, we found that disturbance had no effect on moose cortisol concentrations. Finally, wolves are adaptable to changes in their environment (Mech 1995), but could be affected by disturbance that disrupts their social structure and relationships (Haber 1996; Bryan et al. 2015). We found that wolf cortisol concentrations increased following increased harvest pressure.

Boreal woodland caribou are threatened under the Manitoba Endangered Species and Ecosystems Act, and populations are at risk of decline. The Manitoba government released Manitoba's Boreal Woodland Caribou Recovery Strategy in 2015, which outlines broad recovery strategies to ensure persistence and recovery of caribou in Manitoba over the next 10 years. Indeed, some outlined strategies include protecting 65 – 80% of suitable caribou habitat within caribou management units, and monitoring effects of disturbance (Manitoba Boreal Woodland Caribou Management Committee 2015). We found that caribou cortisol concentrations increased with greater proportion of intermediate logging in their ranges. Logging practices were terminated in 2009 in our study region, but could pose a threat to caribou in the future if industry

returns. Establishment of protected areas would be ideal for minimizing logging development in regions with high caribou densities. If disturbance occurs, it could be grouped to reduce its overall impact on caribou habitat (Courtois et al. 2007; Schaefer and Mahoney 2007; McCarthy et al. 2011). Indeed, large unfragmented coniferous stands > 250 km^2 and > 50 years would be ideal caribou habitat to preserve (Courtois et al. 2007; Vors et al. 2007). Planting coniferous seedlings in disturbed regions could also be important for creating future caribou habitat and minimizing caribou overlap with other ungulates, predators, and hunters (James et al. 2004; McCarthy et al. 2011).

Moose populations declined by 65% between 2000 and 2010 in southeastern Manitoba (Leavesley 2010). Although disturbance had no effect on moose cortisol concentrations, other factors, such as parasitism, predation, and hunting could be important. Gasaway et al. (1983) emphasized the importance of examining additive effects of predation, parasitism, hunters, weather, etc. on moose decline. The Manitoba government has taken several steps to mitigate moose decline in game hunting area (GHA) 26, such as extending deer muzzle loader and rifle hunting seasons, increasing bag limit to three deer per hunter, monitoring parasites and disease in deer and moose, enacting the wolf incentive program ($250 incentive per trapped wolf), increasing the bag limit to two wolves per hunter, extending wolf hunting and trapping seasons, suspending licensed moose hunting, closing rights-based moose harvest in 14% of GHA 26, and removing some human access from roads and trails in areas of high moose density. Moose populations increased by 37% between 2010 and 2013 (Manitoba Conservation and Water Stewardship, unpubl. data), but uncertainty was high and additional years of moose monitoring are necessary to determine whether mitigation measures have been effective.

Fire and logging disturbances provide important early successional forage for moose (Monthey 1984; Gasaway et al. 1989; Rempel et al. 1997). In our study area, logging was terminated in 2009 and fires are suppressed, so loss of suitable habitat for moose could be a future concern. Studies suggest that interspersion of mature habitat with clear-cut areas provide important protection and forage for moose (Courtois et al. 2002; Dussault et al. 2005). However, logging development and wildfires could negatively affect caribou in our study region (Mahoney and Virgl 2003; Schaefer and Mahoney 2007; Courtois et al. 2007; Hins et al. 2009), so impacts on caribou should also be considered when managing moose habitat.

Other important factors that could affect caribou and moose stress response in our study region include black bears (*Ursus americanus*). Little is known about the impact of bear predation on caribou and moose; thus, examining bear diet, particularly in spring and summer when caribou and moose calves are born, could be beneficial. Future studies could also examine the effects of climatic variation on caribou and moose stress responses, particularly since climate warming can cause extreme and unpredictable weather events (Vors and Boyce 2009). Indeed, deep snow and freezing rain can affect access to forage (Vors and Boyce 2009). Changes in plant and insect phenology, such as earlier green-up and greater emergence of insects due to warmer temperatures, could also have negative consequences on caribou and moose (Vors and Boyce 2009).

Wolf populations are stable in eastern Manitoba, even following enactment of the wolf incentive program in winter 2011-12 (Manitoba Conservation and Water Stewardship, unpubl. data). Nonetheless, we found that wolf cortisol concentrations increased following increased harvest pressure. Wolf control is a management strategy that has been used to mitigate ungulate decline (Gasaway et al. 1983; Gasaway et al. 1992; Vors and Boyce 2009). Wolf culling, in

particular, is considered controversial and has received a lot of media attention and public criticism (Mech 1995). Some studies argue that wolf predation maintains low prey densities, so wolf control is required for mitigating ungulate declines (Gasaway et al. 1983; Mech 1995; Boertje et al. 1996). In fact, Gasaway et al. (1983) found that moose and caribou densities increased following a wolf reduction program in Alaska. Alternatively, studies argue that wolf control could affect wolf social structure and dynamics (Haber 1996). Indeed, Haber (1996) suggested that increased harvest of wolves could have lasting effects on size and number of family units, hunting, reproductive, and learning behaviours, and genetic variation. They also suggested that moose kill rates could become more variable with fragmentation of wolf packs.

Some studies do not consider wolf control as an effective long-term management solution for mitigating ungulate decline (Haber 1996; Vors and Boyce 2009). Vors and Boyce (2009) suggested that caribou populations might increase following initiation of wolf culls, but long-term removal of wolves could favour other ungulate species, which would eventually attract wolves to the area. Instead, they suggest that preserving suitable caribou habitat is more important and effective. Effects of wolf control on wolf and prey numbers will vary by system, so each system requires extensive scientific review and careful planning before implementation of control management (Haber 1996). The wolf incentive program in our study region was a short-term (2011-12 to 2015-16) mitigation measure to reduce wolf predation on moose populations. The effectiveness of the program on mitigating moose decline is unknown at this time, but decreased consumption of moose following disruption of wolf social organization (Mocker 2015) could have benefited moose populations.

Overall, measuring cortisol concentrations in hair can be an effective tool for assessing individual health and condition (Cyr et al. 2007). Indeed, we found that moose with high cortisol

concentrations were in poor body condition and were vulnerable to wolf predation. The stress response of wildlife has also been recognized as an important indicator of population decline (Creel et al. 2002; Wikelski and Cooke 2006). Thus, continued monitoring of boreal woodland caribou, moose, and wolf cortisol concentrations with changes in disturbance, climate, and mitigation measures could be an important management tool.

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Appendix A: Validation of collection and processing methods for cortisol and stable isotope analyses of hair

Introduction

Methods of collecting and processing (i.e., washing, homogenizing, etc.) hair could have varying effects on estimates of cortisol concentrations and stable isotope ratios in free-ranging mammals. Some studies have validated procedures for measuring cortisol concentrations in free-ranging mammal hair, but it is still considered a novel technique (Davenport et al. 2006; Macbeth et al. 2010). Alternatively, stable isotope analysis is a common method employed in diet reconstruction analysis (Szepanski et al. 1999; Kelly 2000; Roth 2002, 2003; Drucker et al. 2010; Derbridge et al. 2012), but few studies have validated sample processing procedures in the lab. Thus, we conducted various validation tests to determine the most appropriate methods for sampling and processing free-ranging boreal woodland caribou (*Rangifer tarandus caribou*; hereinafter referred to as caribou), moose (*Alces alces*), and wolf (*Canis lupus*) hair for cortisol and stable isotope analyses.

Body region sampling

Cortisol concentrations could vary by body region, particularly since hair moults at different time periods across the body. Macbeth et al. (2010) found that cortisol concentrations were greatest in the neck region of grizzly bears (*Ursus arctos*), and suggested that the bears might have experienced poor food availability at the time of hair growth in the neck region. Alternatively, structural or functional (e.g., higher glandular cortisol secretions) features of neck hair might have also resulted in higher cortisol concentrations. We wanted to determine whether cortisol concentrations varied across the body, so we measured cortisol concentrations (see Chapter 1, 2, or 3) of hair sub-sampled from the abdomen, shoulder, rump, neck, and leg of two wolf carcasses.

Cortisol concentrations tended to be highest in the neck region (Figure A.1), but due to a small sample size, we cannot conclude much from these results. Nonetheless, we still acknowledge that body region can affect cortisol concentrations, particularly since Macbeth et al. (2010) found differences in cortisol concentrations between body regions of grizzly bears. For our study, caribou hair was typically sub-sampled from the rump region. Wolf samples were submitted by hunters and trappers, so the region of hair collection was unknown. Similarly, moose hair samples were collected from wolf-kill and road-kill sites, hunters, or collared individuals, so the region of hair collection was unknown. To avoid potential body region bias, we tried to measure cortisol concentrations in hair of similar length (i.e., hair would have growth at a similar time).

Methanol vs. water washes

It is important to choose a wash solvent that effectively removes external contamination (e.g., blood, feces, urine, etc.) from hair. Many studies have used alcohols, such as methanol or isoproponal to remove external cortisol contaminants from hair (Macbeth et al. 2010; Ashley et al. 2011; Bechshoft et al. 2011) or 2:1 chloroform/methanol to remove external carbon and nitrogen contaminants (e.g., oils) (Drucker et al. 2010; Derbridge et al. 2012). Water has also been used to wash free-ranging mammal hair prior to stable isotope analysis (Roth and Hobson 2000; Roth 2002, 2003), but is not commonly used to wash hair prior to cortisol analysis. In fact, Davenport et al. (2006) tested and rejected water as a washing agent after finding inconsistent cortisol concentrations among trials, and suggested that water could be penetrating the hair shaft, increasing the risk of extracting internal cortisol while washing. Since few studies have examined how cortisol concentrations and stable isotope ratios vary between methanol and water washes, we wanted to compare their effectiveness.

We divided moose (n = 15) and wolf (n = 14) guard hair samples in half, washed one half with methanol (two 3 min washes with 0.1 mL of methanol/mg hair) and the other with soapy water (one 30 sec wash with 0.1 mL of soapy water/mg hair followed by a rinse), and compared their cortisol concentrations (log-transformed in moose) using a paired t-test. For stable isotope analysis, we divided wolf guard hair (n = 14) and underfur (n = 14) into three parts and washed hair with 2:1 chloroform: methanol (24 hour soak); soapy water wash (1 - 2 mins) and rinse; or a soapy water wash (1 - 2 mins) and rinse followed by 2:1 chloroform: methanol soak. For the third treatment, we dried samples in drying oven for 24 hours between soapy water and chloroform: methanol washes. We conducted Wilcoxon rank-sum tests while blocking for individuals to compare methanol and water washes for guard hair nitrogen and underfur carbon stable isotope ratios (not normal after transformation). We conducted one-way ANOVA tests while blocking for individuals to compare methanol and water washes for guard hair carbon and underfur nitrogen (log-transformed) stable isotope ratios.

There was no difference in hair cortisol concentrations between methanol and water washes for moose (t_{14} = -1.63; p = 0.13) and wolves (t_{13}= -1.98; p = 0.07; Figure A.2). However, cortisol concentrations tended to be higher when moose and wolf hair were washed with water, which could suggest that the duration of the soapy water wash was not long enough to remove external cortisol let alone extract internal cortisol. Future studies should examine how cortisol concentrations vary with various durations of soapy water washes. For the purpose of our study, we continued to wash hair with methanol since it effectively removes blood, urine, and feces contaminants from free-ranging mammal hair (Macbeth et al. 2010).

Carbon stable isotope ratios of wolf guard hair differed among washing treatments ($F_{2, 24}$ = 4.67; p = 0.019; Figure A.3a). In particular, carbon signatures were lower when guard hair was

washed with 2:1 chloroform: methanol compared to soapy water ($p = 0.014$). Nitrogen stable isotope ratios of guard hair ($\chi^2 = 3.60$; $p = 0.165$), and carbon ($\chi^2 = 1.72$; $p = 0.424$) and nitrogen ($F_{2, 24} = 1.94$; $p = 0.165$) stable isotope ratios of wolf underfur did not differ between washing treatments (Figure A.3b). Paritte and Kelly (2009) found that carbon and nitrogen stable isotope ratios of Japanese quail (*Coturnix japonica*) feathers were enriched when washed with 2:1 chloroform: methanol compared to detergent, and suggested that detergents can leave behind residues, affecting feather isotopes. We found that wolf guard hair carbon signatures were higher when washed with soapy water. Water alone might not be removing hydrophobic materials, such as oils; therefore, carbon stable isotope ratios could become inflated if hair is not thoroughly washed (Knoche 2004). Nonetheless, we continued to wash samples with soapy water, but conducted thorough rinses and multiple washes for highly contaminated samples.

Number of methanol washes

The degree of contamination can vary by sample, so it is important to determine the number of methanol washes required to remove external cortisol (i.e., blood, saliva, urine, and feces) from hair without affecting internal cortisol concentrations (Davenport et al. 2006; Macbeth et al. 2010). We classified samples into two categories based on their contamination status (Macbeth et al. 2010). If less than 25% of hair was contaminated with blood and feces, samples were sorted into Category 1; otherwise, samples were placed into Category 2 (26 - 100%). The majority of hair samples (about 80%) fell into Category 1. To determine the number of washes required to remove external cortisol, we measured cortisol concentrations of the wash solutions. We randomly selected $n = 2$ Category 1 caribou hair samples (no samples fell into Category 2), and $n = 5$ Category 1 and 2 moose and wolf hair samples. We then washed all hair samples five times with 0.1 mL of methanol/mg hair for 3 minutes/wash on a shaker table, and

collected 2 mL of each wash solution. We dried the wash solutions using a concentrator, reconstituted the extract with 0.4 mL of radioimmunoassay (RIA) buffer, and measured their cortisol concentrations (see Chapters 1, 2, or 3 cortisol methods). We considered external cortisol to be removed when the cortisol concentration of a wash was lower than cortisol concentrations of previous washes. Alternatively, we considered internal cortisol to be extracted when the external cortisol concentration of a wash was higher than concentrations of previous washes (Macbeth et al. 2010).

After two washes, cortisol concentrations were typically lower or right around the minimum detectable limit (0.05 ng/mL) (Figure A.4). Macbeth et al. (2010) found that a single wash removed > 80% of external cortisol and nine washes did not affect internal cortisol concentrations. Since cortisol concentrations from the second wash hardly differed from cortisol concentrations in subsequent washes, we considered two washes to be sufficient for removing external cortisol for all contamination categories and species.

Methanol extraction rinses

Cortisol has been typically extracted from hair using methanol (Davenport et al. 2006; Macbeth et al. 2010; Ashley et al. 2011). Indeed, some studies have added 1 mL of methanol per 50 mg hair over 24 hours, followed by two additional rinses to ensure cortisol was sufficiently extracted (Macbeth et al. 2010; Ashley et al. 2011). We wanted to determine whether two additional rinses in fact extracted more cortisol, so we pooled a subset of hair samples for moose and wolves, and rinsed the samples zero (n = 4 per species), one (n = 4 per species), or two times (moose: n = 4; wolves: n = 2). For samples receiving one or two additional rinses, we added another 1 mL of methanol, vortexed them (30 sec), centrifuged them for another 15 minutes (4500 rpm at 20°C), and pooled the supernatants. We then dried the pooled supernatant in a

concentrator, reconstituted samples with 0.7 mL of RIA buffer, and measured cortisol concentrations (see Chapter 1, 2, or 3 cortisol methods).

The initial cortisol extraction (no additional rinses) yielded 71.9 and 65.7% of the total extracted cortisol for moose and wolves, respectively. The first rinse yielded an additional 17.8 and 24.0%, and the second rinse yielded an additional 10.2 and 10.4% for moose and wolves, respectively. Using a repeated-measures ANOVA, we found that the number of rinses differed for moose ($F_{2, 6} = 67.31$; $p < 0.0001$). In particular, one ($p = 0.017$) and two additional rinses ($p = 0.004$) differed from zero rinses (Figure A.5). Wolf cortisol concentrations also exhibited a similar trend, but since we only had two measurements for the second rinse (other measurements were below the detection limit of the assay), we did not have sufficient degrees of freedom to run a repeated measures ANOVA. Nonetheless, we continued to rinse all samples two additional times to ensure that internal cortisol was sufficiently extracted from hair.

Conclusions and recommendations

We did not have a large enough sample size to examine the effects of body region on hair cortisol concentrations, but we recognize that cortisol deposition can vary across body regions. Carbon stable isotope ratios in wolf guard hair were higher following soapy water washes compared to methanol washes, so we recommend thoroughly rinsing hair with high-pressure water to remove all contaminants and residues. For hormone analysis, we recommend at least two methanol washes to remove external contamination from hair, and two additional methanol extraction rinses to sufficiently extract cortisol from hair. Overall, we suggest conducting validation tests to determine the most effective sampling and processing methods for measuring cortisol concentrations and stable isotope ratios in hair.

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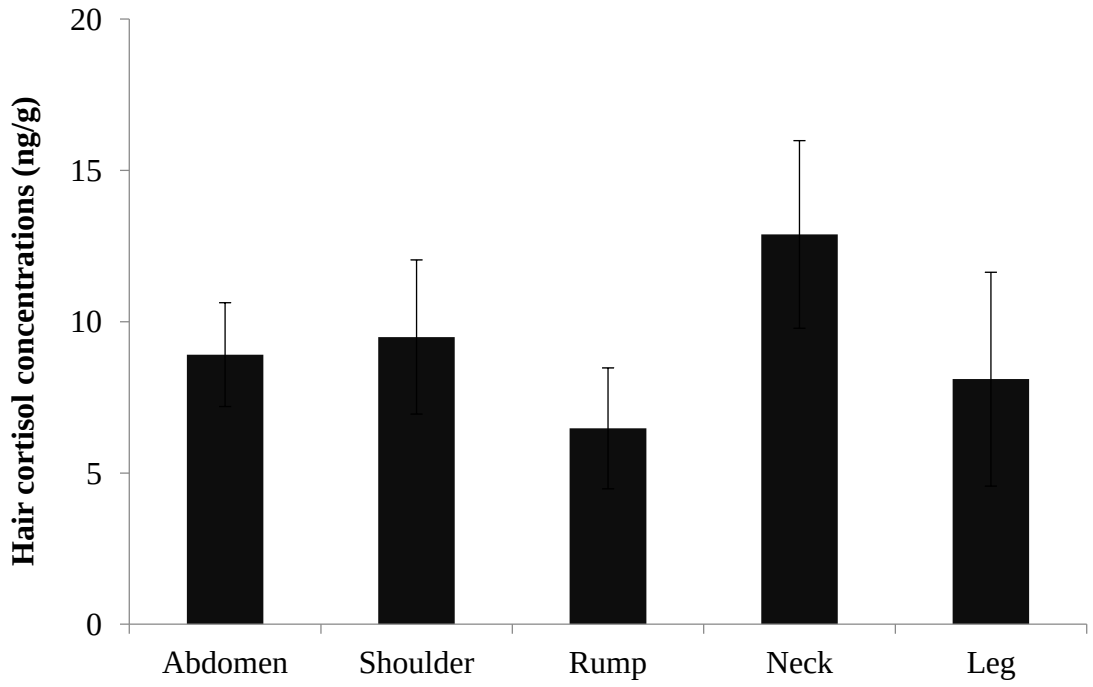

Figure A.1. Cortisol concentrations (mean ±SE) in guard hair from the abdomen, shoulder, rump, neck, and leg of two wolves (*Canis lupus*).

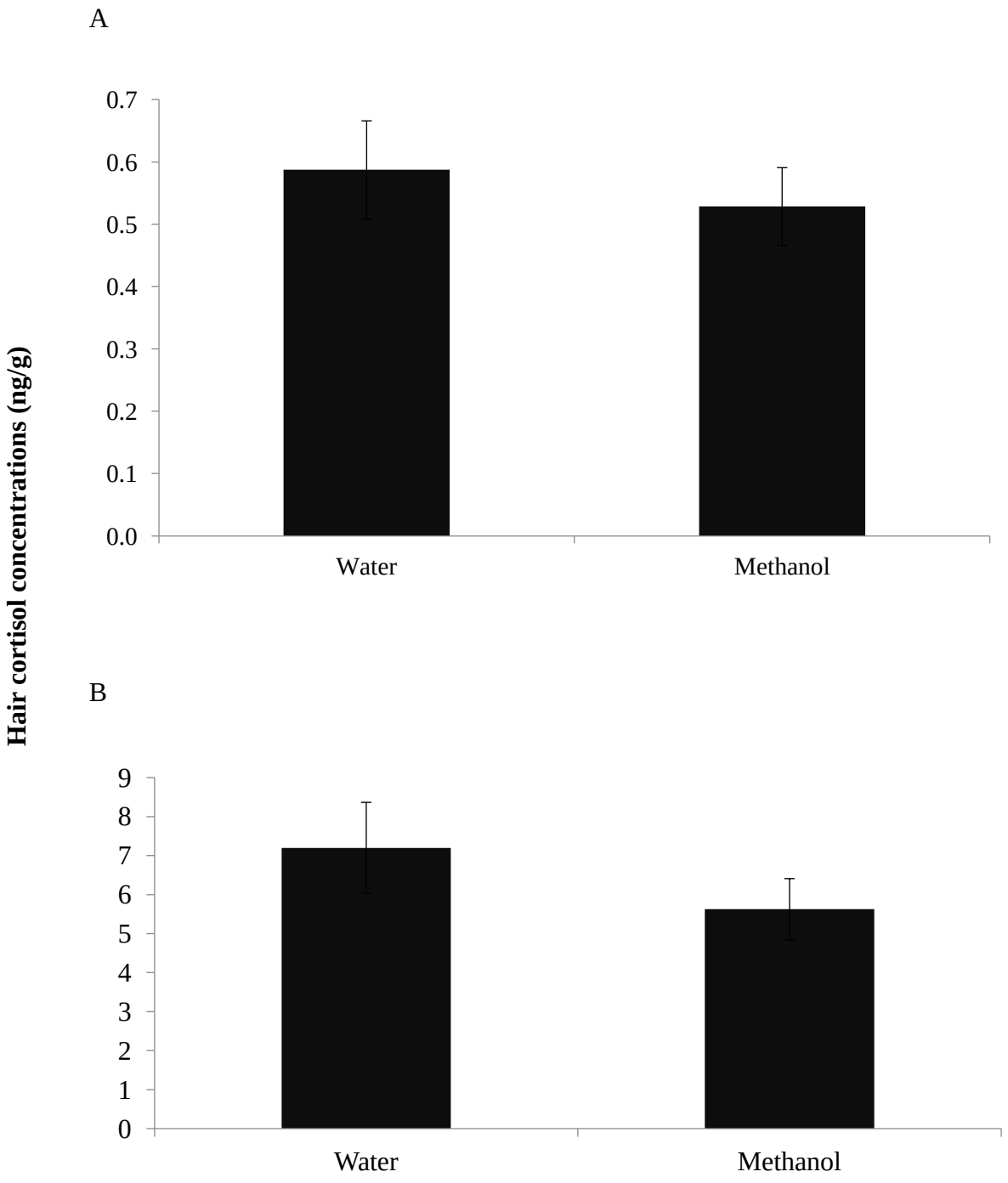

Figure A.2. Cortisol concentrations (mean ±SE) of (A) moose (*Alces alces*; n = 15; log-transformed) and (B) wolf (*Canis lupus*; n = 14) hair washed by soapy water and methanol.

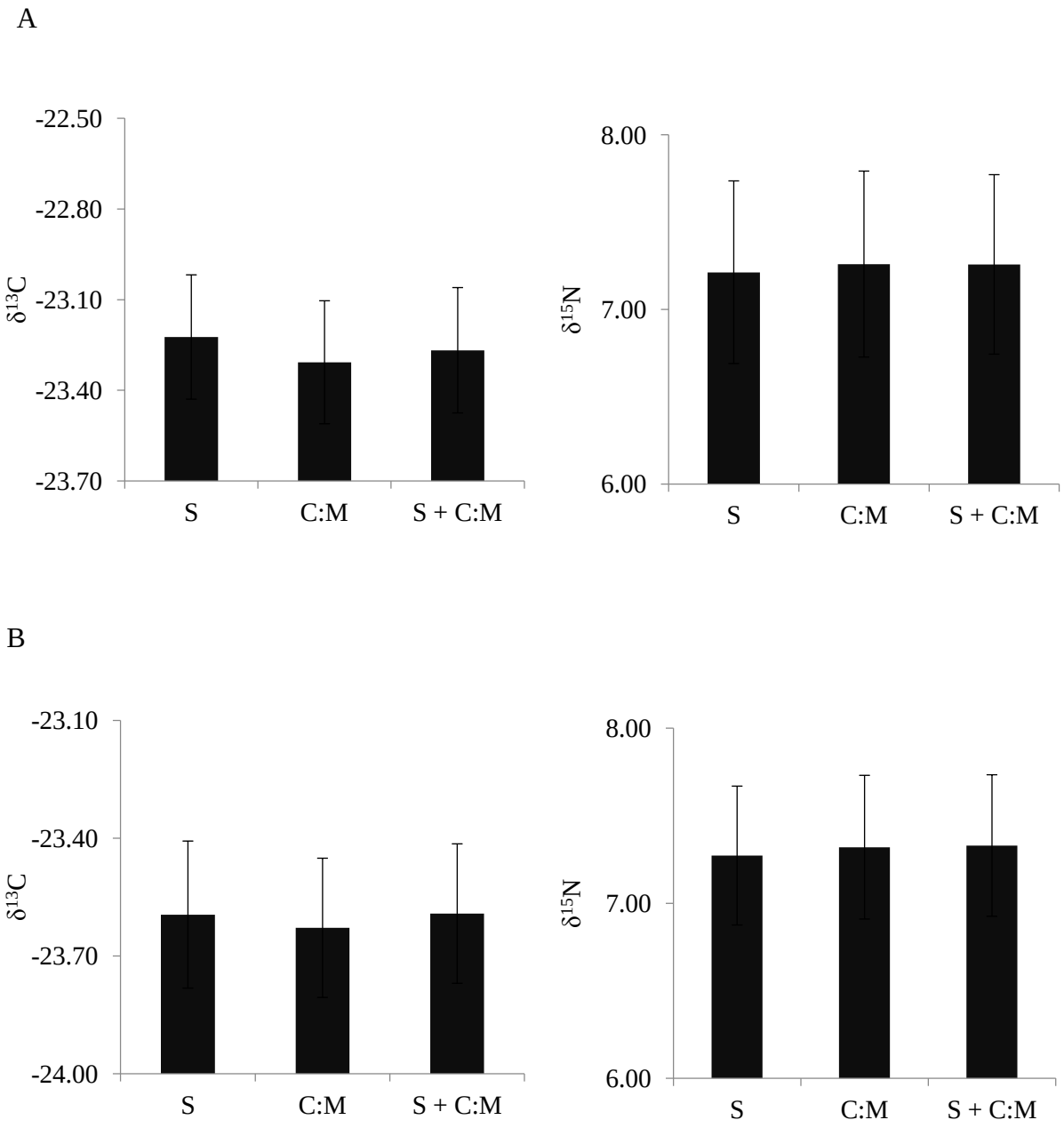

Figure A.3. Carbon and nitrogen stable isotope ratios (mean ±SE) of wolf (*Canis lupus*) (A) guard hair and (B) underfur washed with soapy water (S), 2:1 chloroform: methanol (C:M), or soapy water followed by 2:1 chloroform: methanol (S + C:M).

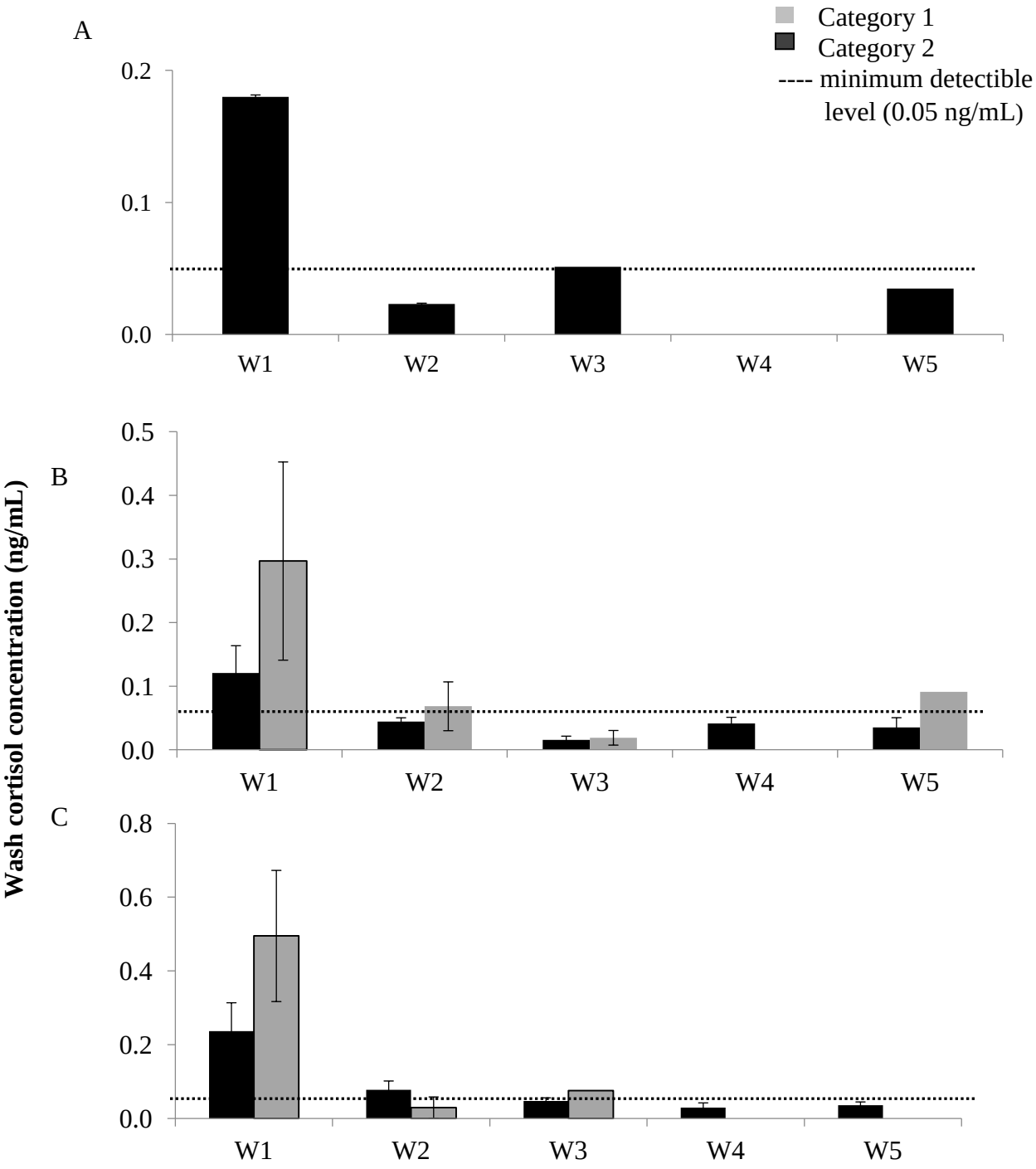

Figure A.4. Cortisol concentrations (mean ± SE) of (A) boreal woodland caribou (*Rangifer tarandus caribou*) (n = 2), (B) moose (*Alces alces*) (n = 5 per category), and (C) wolf (*Canis lupus*) (n = 5 per category) hair exposed to five 3 min washes with 0.01 methanol/mg hair. Category 1 samples were 0 - 25% contaminated and Category 2 samples were 26 - 100% contaminated.

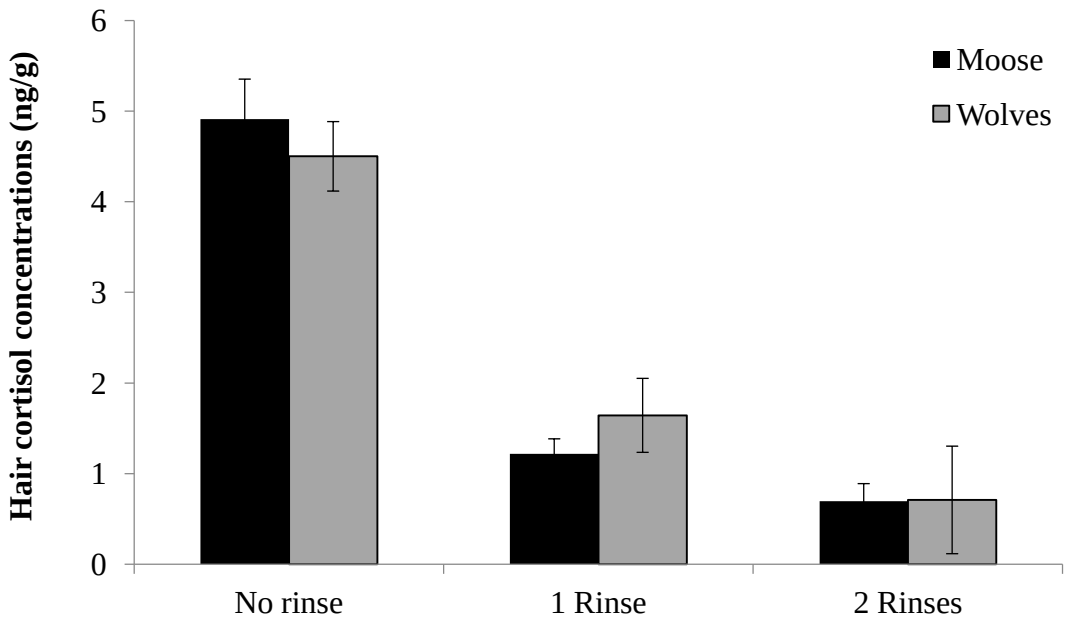

Figure A.5. Cortisol concentrations (mean ±SE) of moose (*Alces alces*) and wolf (*Canis lupus*) hair rinsed zero (n = 4 per species), one (n = 4 per species), or two (moose: n = 4; wolf: n = 2) additional times with methanol (1 mL/50 mg hair).

Appendix B: Physiological stress and body condition of moose in southeastern Manitoba

Introduction

Moose (*Alces alces*) populations in southeastern Manitoba declined by 65% between 2000 and 2010 (Leavesley 2010). Potential influences on moose population fitness that may have contributed to this decline include parasitism, hunting pressure, predation, disturbance, reduced habitat quality, and climate change (Kunkel and Pletscher 2000; Crichton et al. 2004; Lankester 2010; Shura and Roth 2013). Chronic (long-term) exposure to these factors can provoke a stress response in moose that affects their ability to fight disease, reproduce, and survive (Boonstra and Singleton 1993; Charbonnel et al. 2008). To mitigate further moose population decline, it is important to understand the physiological mechanisms affecting their health.

Bone marrow fat has been used to assess body condition in many large mammals (Neiland 1970; Mech and Delgiudice 1985; Murray et al. 2006). A starving animal uses fat stores under the skin and around its viscera for energy (Cheatum 1949; Mech and Delgiudice 1985). After the animal has exhausted these fat stores, it draws upon marrow fat as a last resort (Cheatum 1949). A deer (*Odocoileus virginianus*) can survive 1 to 2 days off marrow fat before dying (Mech and Delgiudice 1985). When marrow fats become depleted, the marrow core appears gelatinous (i.e., more water than fat) and/or red or yellow in colour (i.e., low red blood cell formation and anemia, respectively; Cheatum 1949).

Understanding the relationship between moose stress response and body condition is important for assessing individual health. Studies have found a negative relationship between stress hormone concentrations and body condition in rodents (Charbonnel et al. 2008), lagomorphs (Boonstra and Singleton 1993; Boonstra et al. 1998), and birds (Smith et al. 1994; Heath and Dufty 1998; Rich and Romero 2005). Moreover, high hormone concentrations have

been suggested to contribute to population decline (Boonstra and Singleton 1993; Boonstra et al. 1998; Charbonnel et al. 2008). Indeed, Charbonnel et al. (2008) found that cortisol concentrations increased in the declining phases of vole (*Arvicola scherman)* population cycles, and were negatively correlated with vole body condition and immunocompetence.

We evaluated the link between chronic stress and body condition in moose to provide insight into using measures of stress hormones in hair to evaluate individual body condition and assess population response to changes in disturbance, climate, and management strategies. Our objective was to determine how chronic stress related to moose body condition in southeastern Manitoba. We hypothesized that chronic stress would reflect moose body condition, and predicted that moose with higher cortisol concentrations would have gelatinous, and red or yellow bone marrow cores, suggesting that marrow fat stores are depleted and individuals are in poor body condition (Cheatum 1949).

Methods

We collected moose guard hair (n = 10) from wolf (*Canis lupus*) kill sites in game hunting area (GHA) 26 (see Figure 3.1) in October 2014 and February - April 2014 and 2015. For each moose, we also assessed texture and colour of the marrow core of a femur if available, or otherwise a different limb bone (i.e., tibia, humerus, radius, or ulna). We considered individuals to be in good to marginal body condition (n = 7) when marrow cores appeared solid and white/pink (Cheatum 1949). We specify good to marginal body condition because some moose could be wounded or malnourished (i.e., marginal condition), but still have not exhausted their marrow fat (Mech and Delgiudice 1985). We categorized individuals to be in poor body condition (n = 3) when their marrow cores appeared solid to gelatinous, and red or yellow (Cheatum 1949).

Cortisol becomes integrated into guard hair as it grows, so the stress response of moose would be reflected between summer and fall 2013 – 2014 (approximately June – late September; Daniel Dupont, Manitoba Conservation and Water Stewardship, personal communication) prior to hair collection and marrow core assessments in late fall – early spring 2014 and 2015. We measured cortisol concentrations in moose guard hair using methods described in Chapter 2.

The direction of the causal relationship between physiological stress and body condition in moose (i.e., whether cortisol concentrations affect body condition or vice-versa) is unknown. Since incorporation of stress hormones into hair preceded hair collection and bone marrow core assessments, we used a logistic regression to examine the effects of moose cortisol concentrations on our binary index of body condition (marginal/good vs. poor). We assessed marrow cores in adults only because calves may have marrow cores that appear red until they put on fat stores for winter (Cheatum 1949). All analyses were completed using JMP® Version 12 (SAS Institute Inc., Cary, North Carolina, USA) using a significance level of $\alpha = 0.05$.

Results and discussion

We found that moose with high cortisol concentrations were in poor body condition ($\chi^2_1 = 12.22$; $p = 0.0005$; Figure B.1). These results support our hypothesis, and concur with studies on voles (Charbonnel et al. 2008), snowshoe hares (*Lepus americanus*) (Boonstra and Singleton 1993; Boonstra et al. 1998), and birds (Smith et al. 1994; Heath and Dufty 1998; Rich and Romero 2005).

Whether poor body condition provokes a stress response or is a response to chronic stress is unknown. Some studies have experimentally manipulated hormone concentrations and body condition to determine their effects on one another (Heath and Dufty 1998; Rich and Romero 2005). Heath and Dufty (1998) found that American kestrels (*Falco sparverius*) in poor body

condition responded to capture stress more slowly and had long-term elevated corticosterone concentrations compared to individuals in good body condition. Alternatively, Rich and Romero (2005) found that experimental increases in corticosterone concentrations reduced body weight of European starlings (*Sturnus vulgaris*). Indeed, continuous circulation of stress hormones can affect health of an organism through muscle wasting, growth suppression, immunosuppression, inhibition of reproduction, and poor body condition (Boonstra and Singleton 1993; Boonstra 2004; Charbonnel et al. 2008). Similar to our study, Charbonnel et al. (2008) examined the relationship between chronic stress and body condition in a wild vole population. They found a negative relationship between vole cortisol concentrations and body condition, and acknowledged that poor body condition and nutritional stress could cause an increased stress response in voles, or chronic stress resulting from perturbation of homeostasis could affect condition of voles.

A number of factors could have affected cortisol concentrations and body condition of moose in our study region, including parasites and disease, anthropogenic disturbance, wolf and black bear (*Ursus americanus*) predation risk, hunting pressure, reduced habitat quality, severe weather, starvation, and/or injury. Murray et al. (2006) found that bone marrow fat was lower for moose that died of natural causes (i.e., parasites, disease, predation, and starvation) than anthropogenic factors, suggesting that malnutrition caused moose mortality. Likewise, Franzmann and Arneson (1976) found that marrow fat content of moose killed by wolves or by accidental factors (i.e., road-kill or shot) were higher than marrow fat content of winter-killed (starved) moose. Indeed, the majority of winter-killed moose in their study had marrow fat content of < 10%. The poor-condition moose in our study had solid to gelatinous, and red or yellow marrow cores, which signify < 10% fat content (Cheatum 1949). Factors causing

starvation, including harsh winters, reduced habitat quality, and parasites and disease, could be contributing to poor condition and high cortisol concentrations in moose in our study region.

Our results demonstrate a link between high cortisol concentrations and poor body condition in moose. Further research is needed to explore the causal direction between physiological stress and body condition in moose, and the factors that affect moose condition in our study region. Overall, measuring cortisol concentrations in moose hair can be used to assess population health and recovery in the future, particularly with implementation of mitigation strategies and changes in disturbance and climate.

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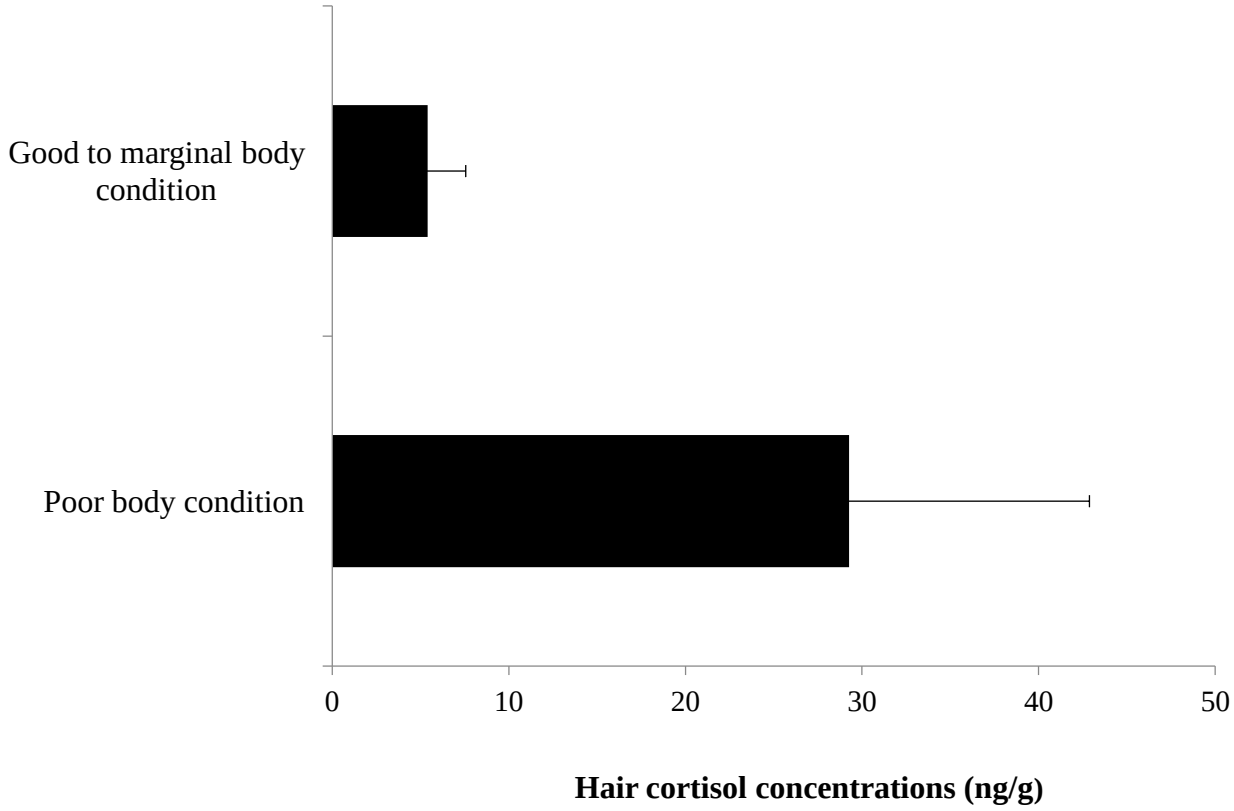

Figure B.1. Relationship between cortisol concentrations and body condition in moose (*Alces alces*) from 2013 to 2014 in southeastern Manitoba (n = 10).